The Influence of Key Variables on the SODIS Method for Application in Kiribati

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"I te timatanga te Kupu, a i te Atua te Kupu, ko te Atua ano hoki taua Kupu"

Hōne 1:1

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"I can do all things through Christ who strengthens me"

Philippians 4:13

Abstract

Globally, children are dying at the rate of nearly one million deaths per year from diarrhoea and dysentery. These diseases are linked to both poor sanitation and contaminated drinking water. In the Pacific Ocean, Kiribati has the second highest rate of under-five mortality (World Health Organization, 2015). This is unlikely to improve with growth rates of 4.5% and an over-stressed water resource (Lal, 2014). One solution may lie in the use of solar disinfection or the SODIS method. The SODIS method has been used effectively around the world to disinfect drinking water since the late 1980s but has only recently been applied in the Pacific. The method involves filling a plastic bottle with water and lying it in the sun all day where upon the water is safe to consume. With its location straddling the equator, Kiribati is ideally placed with a year-round abundance of solar radiation. This free resource can be utilised to improve the quality of drinking water and ultimately reduce child mortality.

The aim of this research was to assess the influence of variables specific to Kiribati on the SODIS method. Experiments were conducted in a controlled environment in the Environmental engineering laboratory at the University of Canterbury to simulate the conditions found in Kiribati. The variables investigated included water conductivity, pH, total water hardness, depth of water i.e. bottle size and age of bottles used.

The method involved filling nine polyethylene terephthalate (PET) bottles with de-ionised water before irradiating the bottles using UVA-340 fluorescent lamps. Seawater was added to the de-ionised water to adjust the conductivity, while sodium bicarbonate and calcium chloride were added to fix the pH and increase the hardness respectively. Primary effluent from the Christchurch wastewater treatment plant was employed as the source of the indicator organisms. These were total coliform and *Escherichia coli (E.coli).* After every hour, one bottle was removed and sampled. Most Probable Number (MPN) of pathogens was determined using the Colilert-18 system by IDEXX.

The laboratory-based experiments did not have a strong correlation with real world experiments due to the use of constant irradiance lighting, artificial water and locally sourced pathogens. However, a strong correlation between experimental results carried out in the laboratory was found. The maximum temperature reached by the bottles under the lights was 38 °C; this meant the dominant form of inactivation was optical due to the ultraviolet radiation.

E.coli had the highest inactivation rate of 50 cm^2/kJ even after beginning with a significant lag. This rate was double the inactivation rate of total coliform of 25 cm^2/kJ, which raised concerns

about the usefulness of E.coli as an indicator organism. E.coli clearly demonstrated that the SODIS method works, but may lead to unsafe water being consumed as it had a higher inactivation rate than other pathogens.

The pathogen inactivation rate reduced with increasing pH. There was a statistically significant difference (p=0.05) in pathogen inactivation between experiments carried out at pH = 6.5-6.8 and pH = 8.3. The implication of this for Kiribati (pH = 8.3) is that more time may be required to achieve the same inactivation as a location with lower water pH, subject to the same amount of solar radiation.

The conductivity of the ground water in Kiribati is weather dependant with levels of 400 μS/cm (post-rain) and 900 μS/cm (prolonged drought) being recorded, with the average level = 700 μS/cm. All three conductivity were investigated with no statistically significant differences between the inactivation rates being determined (p=0.05). The implication of this is that any SODIS method developed specifically for Kiribati will be independent of weather.

Increasing total water hardness (> 530mg/L $CaCO_3$) appeared to increase the inactivation rate of *E.coli.* Increasing total water hardness by adding calcium chloride while maintaining specific conductance led to both variables increasing. Therefore, the increase in activation rate could be related to the increase in conductivity or hardness. It is likely the increase in total hardness was responsible however, as they was no statistically significant difference in the pathogen inactivation across the conductivity experiments.

Results from the small bottle experiments to identify the effect of water depth on the SODIS method were at variance with published literature (Dessie et al., 2014; Kehoe et al., 2001). The smaller bottles (Ø = 60mm) performed significantly worse (p = 0.01) than the larger bottles (Ø = 93mm) for inactivation of *E.coli.* The total inactivation after 9 hours was 1.8 Log and 2.4 Log for small and large bottles respectively. The increased wall-thickness was theorized to be responsible for the poor performance. This highlighted the need for pre-experiment physical examination of bottles to ensure compatible results because SODIS bottles are often recycled from a previous use and/or users may prefer a heavier bottle with the idea that it will last longer. This will result in a significant reduction in the inactivation of the pathogens.

PET bottles were artificially aged under ultraviolet lights for 150h, 1000h, 1500h and 3000h. This resulted in a 15% reduction in light transmitted between the 150h sample and the 3000h sample. Further research is needed to quantify how this reduction in light transmission affects the inactivation of pathogens.

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Terminology

4-MUG	4-methylumbelliferyl-β-D-galactopyranoside
ADB	Asian Development Bank
BPA	Bisphenol-A
c	speed of light (~ $300x10^6$ m/s)
C	Pathogen population at given fluence or time
C_0	Initial pathogen population
Ca	Calcium
$CaCO_3$	Calcium Carbonate
$CaCl_2$	Calcium Chloride
E	energy of photons (J)
EAWAG	Swiss Federal Institute of Aquatic Science and Technology
E.coli	*Escherichia coli*
ErioT	Eriochrome Black T
EDTA	ethylenediaminetetraacetic acid
F	Fluence (J/cm^2)
f	frequency of light (Hz)
h	Planck's constant (~6.63×10^{-34} m^2kg/s)
HWTS	Household Water Treatment and Safe storage
IR	Infrared
k_1	Inactivation coefficient (slope of linear approximate line) (cm^2/kJ)
k_2	Y axis intercept of linear approximation line on inactivation plot
λ	wavelength of light (nm)
Log	Logarithmic scale with a base 10
MDG	Millennium Development Goal
Mg	Magnesium
n	number of entries
NAPA	National Adaptation Program of Action

NH_4Cl	Ammonium Chloride
ONPG	ortho-Nitrophenyl-β-galactoside
p	Probability that one outcome is the same as the other
PC	Polycarbonate
PET	Polyethylene terephthalate
pH	Potential of Hydrogen
PUB	Public Utilities Board
PVC	Polyvinyl chloride
R^2	Square of the Pearson Correlation Coefficient
ROS	Reactive-Oxygenated-Species
s	Standard deviation
SC	Specific Conductance (µS/cm)
SE	Standard error
SODIS	Solar Disinfection
TiO_2-K	Titania-impregnated kaolinite
UV	Ultraviolet
UVA-340	Ultraviolet lamp with peak output at 340nm
UVB-313	Ultraviolet lamp with peak output at 313nm
UV-A	Ultraviolet light with wavelength 315 – 400 nm
UV-B	Ultraviolet light with wavelength 280 – 315 nm
UV-C	Ultraviolet light with wavelength 100 – 280 nm
WHO	World Health Organisation
x_i	value of entry i
$\overline{x}$	entry mean

Units

°C	Degrees centigrade
cm	Centimetre
cm^2	Square centimetre
g	Gram
h	Hour
Hz	Hertz (cycles/s)
J	Joule
K	Kelvin
kJ	Kilo Joule
L	Litre
m^2	Square metre
µS	Micro Siemens
mg	Milligram
min	Minutes
MJ	Mega Joule
MPN	Most Probable Number
mW	Milliwatt
nm	Nanometre
NTU	Nephelometric turbidity unit
s	Second
W	Watt

1 Introduction

In 2015, there were 663 million people, or 9 percent of the global population who did not have access to an improved drinking water source. This was coupled with 2.4 billion people or 33 percent that did not have improved sanitation facilities (UNICEF & World Health Organisation, 2015). A lack of improved drinking sources and sanitation increases the chances of diarrheal disease through waterborne pathogens. Annually, this has contributed to 1.7 billion cases of diarrheal disease with 760,000 young children dying from the symptoms (Luzi, Tobler, Suter, & Meierhofer, 2016). These figures appear high but they are an improvement from 1990 where 24 percent of the global population was without access to an improved drinking water source and 46 percent were not using an improved sanitation facility. These figures from 1990 were part of the driving force behind world leaders gathering in 2000 to develop the Millennium Development Goals (MDGs). The eight MDGs that were developed would provide the framework for the next 15 years (2000 to 2015) with the aim to address the many dimensions of poverty (United Nations, 2015). Reducing child mortality (Goal 4) and addressing the issues of basic sanitation and sustainable access to safe drinking water (Goal 7C) were included in the MDGs.

Ensuring basic sanitation and access to safe drinking water are the most effective measures to reduce child mortality. Ninety percent of deaths from diarrhoea are linked to these factors (UNICEF & World Health Organisation, 2015). The intention of MDG 7C was to halve the 1990 population without access to basic sanitation and safe drinking water by 2015; this has been met with mixed success. For safe drinking water this target was achieved in 2010 on the global scale whereas by 2015 the sanitation target had still not been reached (UNICEF & World Health Organisation, 2015). The World Health Organization's (WHO) ideal solution for safe drinking water is to have reliable safe piped water to every household. In realisation that this solution may be some time in coming, the WHO have published a book on Household Water Treatment and Safe storage (HWTS) for use in the short term. The HWTS focuses on treating water at its point-of-use rather than at its point of delivery. Basic approaches to treating the water are investigated e.g. disinfection, filtration and assisted sedimentation. The justification behind the HWTS book is that many people do not have a continuous supply of piped safe drinking water, so storing water is essential. This stored water is then subject to possible recontamination (World Health Organisation, 2013). Although the technologies used in the HWTS are not new, what is new for some cultures is the requirement for them to be responsible

for the treatment of their own water. This is a considerable hurdle to the successful implementation of HWTS (World Health Organisation, 2013).

The WHO lists three distinct approaches as being acceptable to HWTS. These are sedimentation, filtration and disinfection. Each of these three approaches can in turn be further broken down into different methods for producing safe drinking water. An example of this is disinfection, which can be further broken down into chlorine disinfection, solar disinfection, ultraviolet (UV) disinfection (using a germicidal wavelength light) and boiling (World Health Organisation, 2013). Clasen, Haller, Walker, Bartram, and Cairncross (2007) looked at the cost effectiveness of different HWTS methods as shown in Figure 1-1.

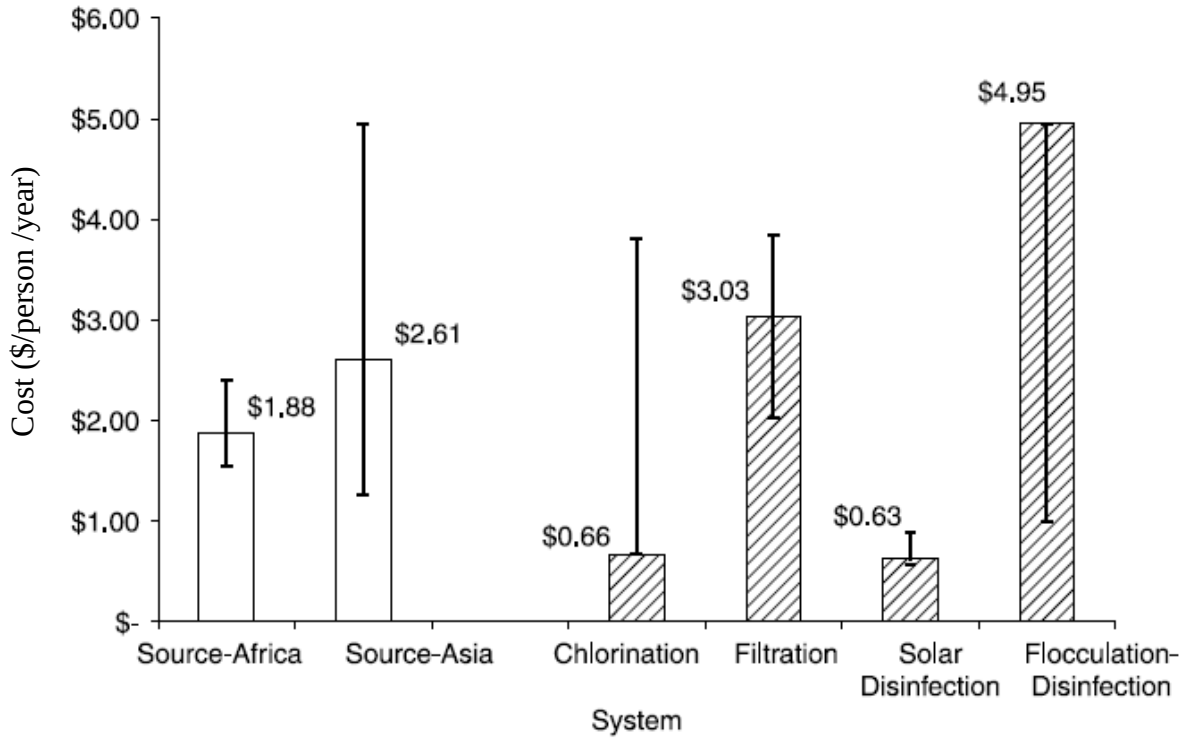

Figure 1-1: Graph of Annual Mean Cost per Person for Treating the Source and Different HWTS Methods

Note: Adapted from (Clasen et al., 2007)pg. 604. Reprinted with permission.

1.1 Solar disinfection - SODIS

Figure 1-1 illustrates that treating the water at the source costs approximately $1.88 to $2.61/person/year depending on the continent. This is nearly three times the cost of the cheapest HWTS method being Solar Disinfection or SODIS at a price of $0.63/person/year. SODIS keeps its cost low, as it does not require a distribution network or chemicals to treat the water. It involves filling a clear plastic bottle with water and placing it in direct sunshine all day whereby the UV component in sunlight disinfects the water. The countries who have not met the MDGs are often the poorest countries with the least amount of money and resources available; therefore, any method that is used needs to be affordable. As the SODIS method has the lowest mean annual cost per person and does not require the establishing of supply networks, it would be the logical method to warrant further investigation.

1.2 Kiribati

For the implementation of the MDGs, the United Nations divided the world into nine regions. In relation to Goal 7C, which aimed to address issues around basic sanitation and safe access to drinking water by halving the proportion of the population without access, four developing regions failed to meet their target. Oceania was the lowest scoring region with only 56% of the population using an improved source of drinking water. This is further highlighted by the National improved water ratio for Oceania

$$= \frac{Population\ gaining\ improved\ water}{Population\ increase} = 0.6$$

A value less than 1.0 indicates that the improvements in water quality are not keeping abreast of the population increase (UNICEF & World Health Organisation, 2015). In the Oceania group, Kiribati had the second highest rate of under-five mortality with 58.2 deaths per 1000 live births. This value was only exceeded by Papua New Guinea with 61.4 deaths per 1000 live births (World Health Organization, 2015).

Kiribati is an equatorial island nation in the central Pacific Ocean. It has a population of 106,000 people spread over 33 islands divided into three groups. These are the Gilbert islands, the Phoenix islands and the Line islands as shown in Figure 1-2 (UNICEF & World Health Organisation, 2015). The islands typically have a height of 0-3m above-mean-sea-level that makes them extremely susceptible to climate change and rising sea levels.

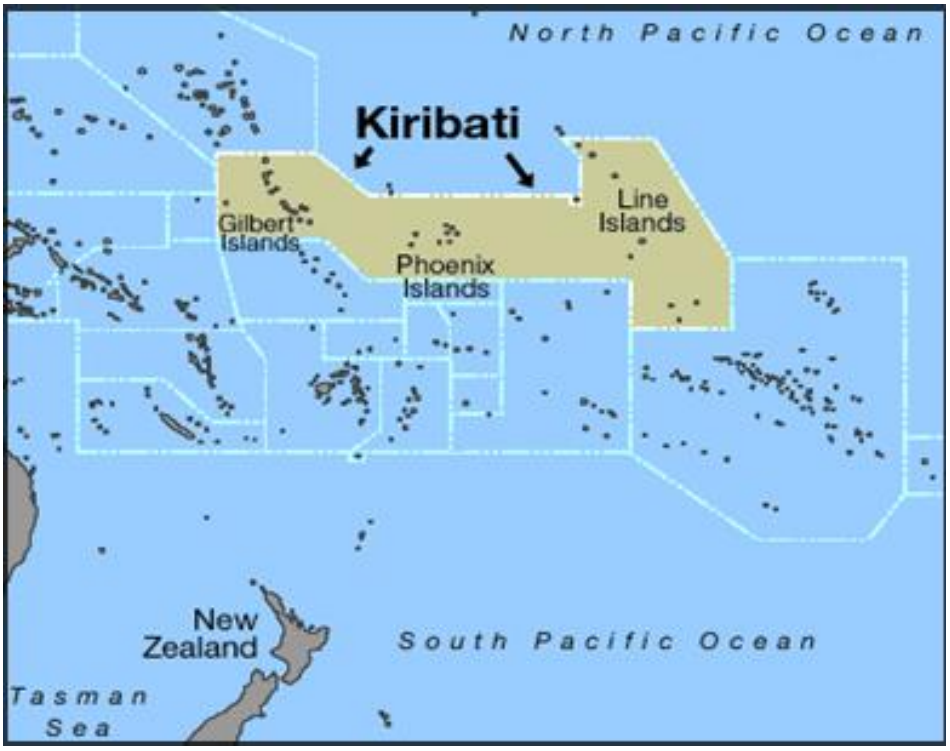

Figure 1-2: Location of Kiribati within the Pacific Ocean. Copyright 2012 by (Office of the President Republic of Kiribati) Reprinted with Permission.

Approximately half the population of Kiribati live in South Tarawa on the Tarawa Atoll, which is located in the Gilbert Islands group as shown in Figure 1-3.

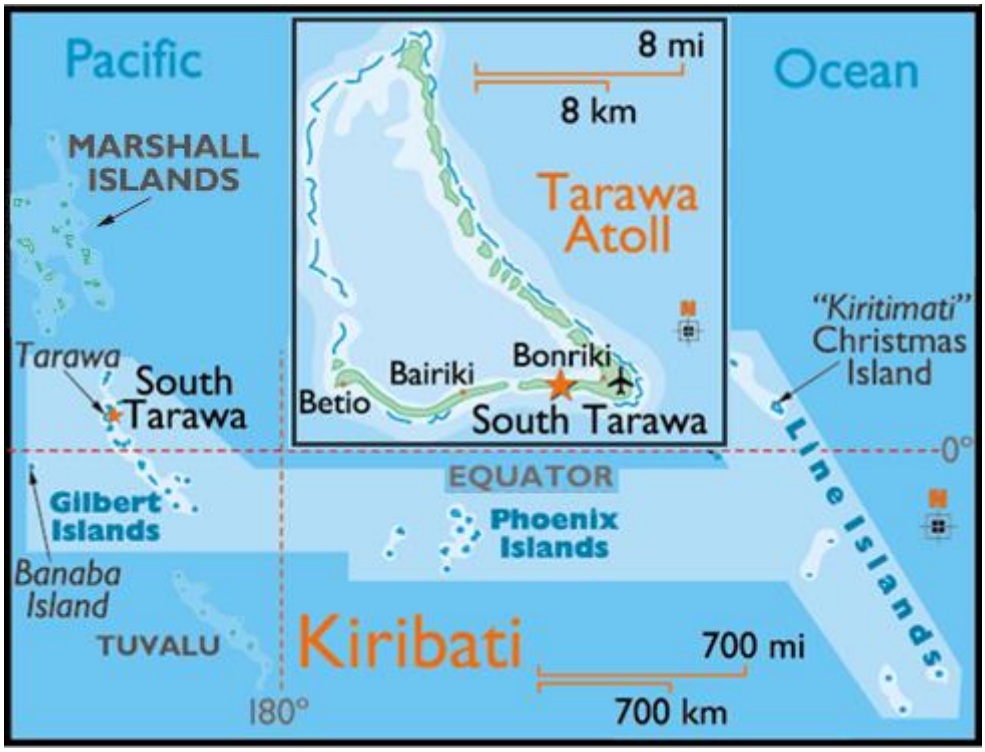

Figure 1-3: Location of Tarawa within the Kiribati Group of Islands. ***Note****: adapted from www.worldatlas.com/webimage/countrys/oceania/lgcolor/kicolor.htm Copyright by GraphicMaps.com*

The average annual rainfall in South Tarawa is in excess of 2000mm. On the surface this seems adequate however this figure fails to take into account the impact of La Niña induced droughts which on average last nearly 2 years (White, 2010). Piped water is supplied to residents in South Tarawa for 2 hours every second day by the Public Utilities Board (PUB) for a flat fee of A$10 / month / household. This fee is less than the cost of service delivery, however the PUB is reluctant to increase the charge to cover the cost as they believe it will negatively impact on households (Lal, 2014). Due to the infrequent supply, residents have to store piped water as well as supplement with other sources of water, typically groundwater. Given the shallow depth of the fresh water lens, the ground water is easily contaminated; and as such, all sources of drinking water including the piped supply need to be treated prior to use (Stärz, 2015). This is where the HWTS information from WHO is essential in ensuring safe drinking water is available for residents. Kiribati with its high rates of annual sunshine hours and ultraviolet radiation is particularly suited to SODIS (Stärz, 2015). Treatment of water with SODIS improves access to safe drinking water and thus the objective under Goal 7C of the MDGs.

2 Literature review

2.1 Drinking water in Kiribati

The South Tarawa Water and Sanitation Roadmap for 2011-2030 was published in 2011 with funding from the Asian Development Bank (ADB). This report investigated the current conditions of drinking water in South Tarawa and found a lack of adequate regulation and control. Poor resource management had resulted in all natural water sources being considered unfit for drinking due to contamination from human and / or animal faeces (Fraser Thomas Partners, 2011). There is a link between high rates of diarrhoea and dysentery diseases in the local communities and contaminated water sources. This was highlighted in 2010 when 54% of the people from the highly crowded area of Betio - South Tarawa were required to visit a medical centre due to diarrhoea and dysentery (Fraser Thomas Partners, 2011). It has been indicated that visiting a medical centre is often the last resort for the local people. Therefore it could be inferred that the rates of diarrhoea and dysentery were actually higher than those reported (Fraser Thomas Partners, 2011).

There are three water sources available to the residents in South Tarawa, rainwater, well water and the PUB piped water. As the PUB water is only available every second day and for only 2hours, residents must use a combination of all three water sources to meet their needs (Lal, 2014). Due to the contamination of well water and rainwater, any improvements to the quality of the public supply will not see any significant health benefits amongst the population. Improvements in public health through upgrades in supplied water quality, will only be achieved with comprehensive community programs to promote awareness of the risks associated with consuming polluted water (Fraser Thomas Partners, 2011).

2.1.1 Factors affecting drinking water

In maintaining high quality drinking water, it is essential that good water resource management practices be put in place (World Health Organization, 2004). The government of Kiribati recognised this in 2004 and drafted the National Adaptation Program of Action (NAPA) which was published in 2007 (McIver, Woodward, Davies, Tibwe, & Iddings, 2014). The ultimate aim of the NAPA was to identify, prioritise and then plan for the issues associated with climate change. The issues ranked first equal were “Water Resource Adaption Project” and “Simple well improvement” (Ministry of Environment Land and Agricultural Development, 2007). These two issues align with “Guidelines to drinking water quality”, which aim to protect the source water from being contaminated by microbial pollution and chemical pollution (World

Health Organization, 2004). Land use plays a significant part in protecting or polluting the water source. Different land use factors that have been identified by the World Health Organization (2004) that are common to Kiribati include:

- The modification of the land cover, including natural cover to urbanized cover and from permeable to non-permeable surfaces.
- The method used for water extraction, e.g. protected / unprotected shallow ground water extraction.
- Residential development and methods for disposing of effluent. This can range from centralized wastewater treatment to open defecation practices.

2.1.2 Health-based targets for drinking water

In any drinking water safety framework, there needs to be health-based targets. When establishing them they should take into account the contribution that drinking water quality makes to the general overall health of the community. McIver et al. (2014) developed a "health risks" matrix for Kiribati based on impact and likelihood. Impacts ranged from "insignificant" to "catastrophic" while the likelihood varied from "rare" to "almost certain". The aim was to identify health issues a) that have strong links to climate change, b) would increase the disease burden or c) could be reduced by practical solutions. One high priority health issue identified was "Water safety and water-borne diseases" (McIver et al., 2014).

2.2 Microbial water quality

Microbial water contamination is the most common and widespread health risk with regards to drinking-water (World Health Organization, 2004). The extent of this burden depends on a variety of factors including who is exposed, what is the pathogen, living conditions and how severe the disease is. Concerning who is exposed, the very young and the very old are more susceptible along with those that already have an impaired immune system. An example of impaired immunity would be someone that has just suffered from diarrhoea or dysentery. The ability to measure and control microbial contamination is essential in minimising the health risk. When pathogens leave their host, the majority immediately begin to lose their viability and infectiousness. High persistence outside a host and the ability to multiply in water are two factors that contribute to pathogens and parasites being more common. Viruses along with cysts, ova and oocysts are all unable to multiply in water.

To minimise the risk of microbial hazards typically health-based targets are employed calculated on a tolerable burden of disease. These are preferred over water quality targets as monitoring water post-treatment is not cost-effective or considered feasible (World Health Organization, 2004). When testing for microbial water quality, *Escherichia coli (E.coli)* is often used as an indicator of contamination by faecal pollution. This is because *E.coli* occurs in the intestines of warm-blooded animals and should not be present in drinking water. *E.coli* does have its limitations however, in that it is less resistant to disinfection than other forms of microbes such as viruses and protozoa. Therefore, the absence of *E.coli* post disinfection does not necessarily mean that the treated water is safe to drink. In situations where this may be an issue bacterial spores and/or bacteriophages can be used as they have a higher resistance to disinfection (World Health Organization, 2004).

Figure 2-1 below shows the relationship between total coliform, faecal coliform and *E.coli*. These are common indicator pathogens used when working with contaminated water. The diagram shows that the detection of total coliforms in a sample does not necessarily mean there are faecal coliform or *E.coli*, as these are only a subset of total coliform. However, detection of *E.coli* confirms the presence of faecal coliform and total coliform in the sample.

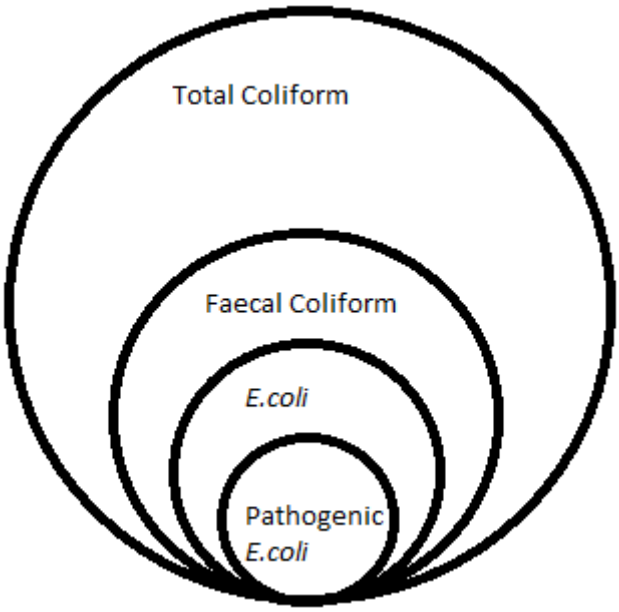

Figure 2-1: The Relationship Between Commonly Used Indicator Bacteria

Note: Adapted from http://www.mybiolumix.com/the-debate-coliforms-fecal-coliforms-and-enterobacteriaceae -as-indicator-organisms/

2.3 The SODIS method

The SODIS method involves filling a transparent container with water from any available source and lying it in full sunlight in an elevated position from morning to evening, where upon the water is safe to consume. The focus of the water treatment is on killing pathogens that can cause diarrhoeal disease; it does not remove heavy metals or pesticides that may be present (Wegelin et al., 1994). The disinfection process is carried out mostly by UV radiation in the 315-400nm bandwidth which indirectly causes damage to the DNA of the organisms (Luzi et al., 2016). The value of SODIS lies in that it is cheap, uncomplicated and most importantly effective. The only equipment needed is a transparent container, typically glass or plastic and sunshine. As there are no ongoing consumables there is no need to establish a supply chain, this makes it attractive for isolated communities and areas of low income. The Swiss Federal Institute of Aquatic Science and Technology (EAWAG) defined the SODIS method in four steps to ensure its effectiveness each time that it is used (Luzi et al., 2016):

1. Wash a plastic bottle.
2. Fill the bottle with water.
3. Expose the bottle to the sun.
4. Consume the water or store in the bottle.

2.3.1 Effectiveness of SODIS on pathogens

Examples from the full suite of pathogens, including bacteria, viruses, protozoa and helminths have been researched to identify their resistance to the SODIS method (Luzi et al., 2016). Due to the different experimental setups and light sources used, it can be difficult to compare their results. Overall the results demonstrate that the SODIS method works well at inactivating bacteria with typical reductions of 4 log being recorded after 1 day of exposure ($1MJ/m^2$) (Luzi et al., 2016). Viruses yielded mixed results with some (bovine rotavirus and bacteriophage) realising a >3log reduction in 3 hours while others (encephalomyocarditis) required >12 hours for a 3 log reduction (McGuigan et al., 2012). Rotavirus which is linked to most virus-related diarrhoea in children, can be transmitted through drinking water but is more often caught through poor sanitation and hygiene (Luzi et al., 2016). The SODIS method worked against the protozoa *Cryptosporidium pavum* with infectivity rates dropping from 100% to 7.5% after 6 hours of exposure (Méndez-Hermida, Castro-Hermida, Ares-Mazás, Kehoe, & McGuigan, 2005). After an additional 6 hours irradiating the infectivity rate dropped to 0%. Heaselgrave and Kilvington (2011) investigated the helminth *Ascaris* and found that the larvae continued to develop after being irradiated using the SODIS method.

2.3.2 Bottle type

There are several plastic bottles used for commercial packaging that could be a source for use in SODIS. The material typically used for the plastic bottles is either polyethylene terephthalate (PET), polyvinyl chloride (PVC) or polycarbonate (PC). The respective plastic recycling numbers used for identification are 1, 6 and 7. To increase the UV longevity of the plastic bottles and the contents, bottle manufacturers use additives in the plastic to slow oxidation. It is these additives that can leach out of the plastic and into the contents causing health concerns (McGuigan et al., 2012). There are two reasons for not using PC for SODIS. Firstly, it can release the known carcinogen bisphenol-A (BPA). Secondly, the chemical/physical structure of PC blocks the transmission of UV light preventing the disinfection process of SODIS occurring. On investigating PET bottles, Wegelin et al. (2001) found that although sunlight does cause a breakdown of the PET, it is on the outside surface of the bottle only. There was no recorded leaching or release of any photoproducts from the PET bottle into the water during the experiments. Further research investigating the leaching of PET bottles was published in 2008. The findings confirmed the results from 2001 that the SODIS method was safe with respect to leaching from PET bottles (Schmid, Kohler, Meierhofer, Luzi, & Wegelin, 2008). PVC is not a good choice for the SODIS method as the ductility enhancing plasticizers can leach out into the water. In addition to having different recycling numbers, PET can be distinguished from PVC using a burn test. PET burns easier and has a sweet smell unlike the pungent smell from PVC. A cut test can also be used to differentiate between PET and PVC, as PVC displays a blueish hue along the cut edge (EAWAG, 1999).

EAWAG recommend the use of PET plastic bottles over glass bottles. Tests carried out by EAWAG on commercial glass bottles produced the same UV transmittance as PET bottles (Luzi et al., 2016). Borosilicate glass shows promise over PET and standard commercial glass as it has a higher transmission of UV light in the UV-B bandwidth. This means it could have higher inactivation rates over the same timeframes when compared to PET (McGuigan et al., 2012). Glass does have drawbacks in that it is heavier, more expensive to produce and transport and it is prone to breakage. Plastic drink bottles are slowly phasing out glass bottles, as they are cheaper to make and transport. The ability to withstand being dropped makes plastic bottles last longer. Scratches and degradation of the plastic outside wall limit the life of the PET bottles through a reduction in the UV transmittance.

2.3.3 Bottle size

The SODIS method involves sunlight penetrating the water within the bottle. As UV intensity diminishes with the depth of the water column, there is a limit to the depth of the water where the method is still viable. An early experiment investigating the difference in inactivation kinetics between a 1.5L bottle with 11cm diameter and a 0.5L bottle with 6.5cm diameter showed that depth had no significant effect (Kehoe et al., 2001). Dessie et al. (2014) carried out experiments with four bottle sizes with depths of 5.5, 7.5, 8.5 and 10cm. The results from those experiments shown in Figure 2-2 clearly illustrate the importance of limiting water depth.

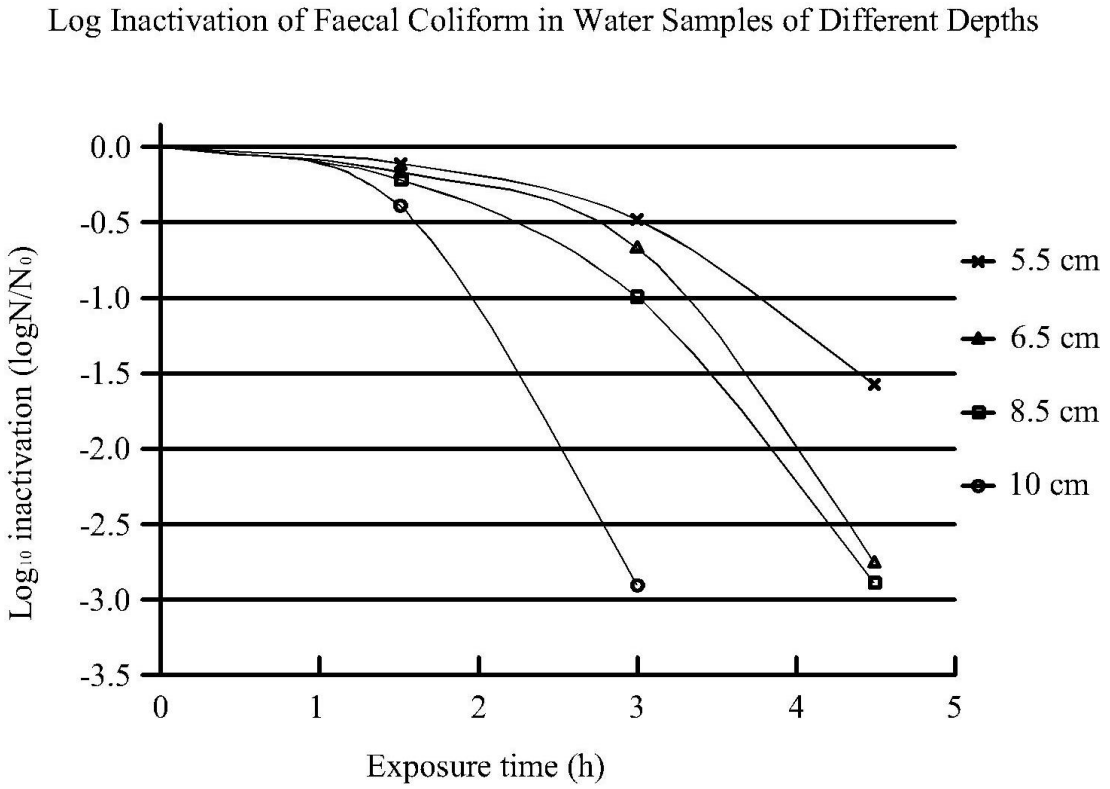

Figure 2-2: Log Inactivation of Faecal Coliform on Water Samples Having Different Water Depth (5.5cm, 7.5cm, 8.5cm and 10cm).

Note: Adapted from (Dessie et al., 2014) p.3, reprinted with permission.

After 3 hours exposure, there was nearly a 3.0 log reduction with the 5.5cm water depth compared with only a ~ 0.5 log for the 10cm depth as seen in Figure 2-2. Mani, Kanjur, Bright Singh, and Reed (2006) went as far as producing their own PET bottle to study the SODIS method. Their rectangular bottle was 9.5cm wide x 5.2cm deep, with a volume of 1125mL and a mass of 48g. This bottle and a commercially available PET bottle were tested for performance and the results compared. The commercial bottle was circular with a diameter of 7.5cm, a 1L

volume and a weight of 22g. Both bottles achieved similar results of pathogen inactivation even with having different depths of water columns. The explanation for this was that the increase in light intensity from the shorter light path of the custom bottle was nullified by the reduced transmission of sunlight through the thicker plastic wall (Mani et al., 2006). In summary both, the depth and the wall-thickness need to be minimised to achieve the best results.

The volume of SODIS bottles should not exceed two litres (Luzi et al., 2016). As discussed, the performance of SODIS decreases with increasing water depth (Dessie et al., 2014). The typical diameter of a 2.0 litre bottle is 10-12cm. It may be possible to use bottles with a higher volume but due to the drop in performance, they may require more hours of irradiation than is available in a standard day. For disinfecting larger volumes, it is more efficient to split it across multiple smaller bottles.

2.3.4 Bottle colour, clarity and cleanliness

Bottles originally designed for other uses are often used for SODIS. Some of these bottles may have had contents they were particularly susceptible to sunlight. To prevent the product breaking down before consumption manufacturers colour the bottles with colorants. As the purpose of these colorants is to reduce the transmittance of light through the plastic these bottles are unsuitable for use with the SODIS method. Light blue bottles tested by EAWAG were the exception in that they transmitted a high percentage of light (Luzi et al., 2016). In order to avoid confusion over what constitutes a light blue bottle, only colour free bottles are recommended for SODIS.

Another means of protecting their product that manufacturers use is by making the bottles opaque. An example of this would be containers used for milk. The lack of clarity makes these bottles unusable for the SODIS method as the opaque finish absorbs light. This prevents it from carrying out the disinfection of the water inside. Only colour free, clear bottles should be used for disinfecting water using the SODIS method.

The cleanliness of the bottle can affect the performance during the SODIS method. Dirt on the outside or inside of the bottle can absorb or reflect light preventing it from entering the bottle. In addition to dirt, contaminants can accumulate on the surface of the bottle, which may be transferred to the water or the hands. Therefore, bottles must be washed prior to using for SODIS to remove any contamination or dirt.

2.3.5 The role of oxygen in SODIS

The SODIS method requires oxygen in the water to create the Reactive-Oxygenated-Species (ROS), which are responsible for the disinfection. The results from experiments carried out by Reed (1997) indicated that aerobic conditions are required for the SODIS method to be fully effective. One method to ensure aerobic conditions is to only fill the bottle two-thirds and then shake vigorously for 30s before filling to the top and capping (McGuigan et al., 2012). In the six-step method published by the Environmental Health Services in Kiribati it is recommended to three quarter fill the bottle before shaking for 10s (Environmental Health Services, 2016). The SODIS manual leaves out the partial filling and shaking recommendation. The reasons being that adding another step complicates the method and that shaking is unnecessary as the water gets oxygenated when it is poured into the bottle (Luzi et al., 2016).

2.3.6 Disadvantages of SODIS

Like all methods for obtaining safe drinking water, SODIS does have its disadvantages. These include time for inactivation and costs due to theft of bottles. However, the main disadvantage is that it is weather dependant. With its reliance on solar irradiation, the SODIS method requires more time with any reduction in direct sunlight. The most common reduction is due to cloud cover. The SODIS manual published by Eawag states that one day irradiation is required for up to 50% cloud cover, two days irradiation for >50% cloud cover and that alternative methods of disinfection should be used on days of continuous rainfall (Luzi et al., 2016). Therefore, countries that experience rainy seasons would have to forgo SODIS during these times.

2.3.7 SODIS additives / accelerants

Improvements to the SODIS method are difficult due to its simplicity. One area for improvement is the time required to inactivate the pathogens. To speed up the inactivation, researchers have looked at water additives and the effect they have on the inactivation time. Additives have included hydrogen peroxide (H_2O_2), titania-impregnated kaolinite, riboflavin (Vitamin B2), semiconductors or naturally occurring items such as lime juice and copper.

In investigating titania-impregnated kaolinite (TiO_2-K), Chong, Jin, and Saint (2011) first measured the pathogen inactivation of their UV-A light source. The UV-A 8W black light achieved less than 1.0 log reduction in pathogen numbers after 300min of irradiation. Following the addition of $Ti0_2$-K under optimum conditions, a 5.0 log reduction was achieved in 120mins. Harding and Schwab (2012), in their research recorded a baseline of 1.5 log reduction with *E.coli* after 30 mins of irradiation. Following this, an addition of lime juice

achieved a 5.6 log reduction. The addition of semiconductors is based on the principle that SODIS disinfects with ROS. When the sunlight strikes the semiconductor, photocatalysis occurs releasing highly oxidative species which carry out the disinfection (Byrne, Fernandez-Ibañez, Dunlop, Alrousan, & Hamilton, 2011).

The drawback of using additives is that it can create an additional cost or step in the SODIS method. The people using the SODIS method may not have the funds or the means to obtain the additives. If an appropriate additive can be sourced or grown locally, then information on the positive benefits of using it should be supplied to communities. An example of this is the *Moringa oleifera* seed, which is native to northwestern India. Ferreira et al. (2011) found that adding ground *Moringa oleifera* seeds bound up colloidal solids thus reducing turbidity in the SODIS water. This increased the performance of the SODIS method by improving the water clarity. Although the *Moringa oleifera* seeds are cultivated globally they may not be available to everyone, this further highlights the problem of people not having the means to obtain the additives. In answer to this problem, Dawney and Pearce (2012) investigated the coagulating properties of table salt (NaCl) which is abundant in the Pacific. Their results were positive with a particle removal efficiency of 98% with samples of suspended bentonite. One concern in adding another step to the SODIS method is that incorrect execution is possible. This could result in the consumption of contaminated water with the belief that it is safe.

Instead of adding another step to improve the performance of the SODIS method, increasing the irradiance can also shorten the time required to disinfect the water. Martín-Domínguez, Martín-Domínguez, Alarcón-Herrera, and González-Herrera (2005) investigated different shaped solar concentrators and their effect on pathogen inactivation. The results showed that the larger solar concentrators achieved the highest inactivation in the least time. The disadvantage of using solar concentrators are that they only work for a particular angle of the sun and need to be relocated for different seasons to account for the change in the sun's path. A simpler method is to locate the SODIS bottle onto a corrugated iron roof. This has several benefits including increasing the irradiation through reflection, reducing the shadows on the bottle and minimising the possible contamination of the bottle from animals and vermin.

2.4 Social acceptance of SODIS

SODIS is a recognised household water treatment method that reduces diarrheal disease (Luzi et al., 2016). It only requires a PET bottle and sunlight and yet the uptake on SODIS is low. McGuigan et al. (2012) claim that the success in using SODIS only comes through change in behaviour. This is because seeing the benefits of the SODIS method requires modification of daily practices. To achieve maximum benefit from SODIS households must exclusively consume SODIS water as other sources of drinking water may be contaminated. Reasons for the poor uptake or continued use of SODIS include non-belief in its effectiveness, belief that it is not required and negative social opinion (Luzi et al., 2016). In a field trial of SODIS with the Maasai people in the Kajiado district Kenya, Conroy, Meegan, Joyce, McGuigan, and Barnes (1999) credit the uptake and continued use of SODIS to the use of Maasai elders as fieldworkers. These elders were educated, informed and respected by the community they were working with. Eawag produced the first SODIS manual in 2002 and then a revised edition in 2016 as a means of educating the community about the effectiveness of SODIS. This paired with regular visits from SODIS promoters has shown the best results rather than flyers or posters only. Promoters often disseminate data on the quality of local drinking water, which reinforces the need for water treatment (Luzi et al., 2016; Meierhofer & Wegelin, 2002).

The issue of negative social opinion is difficult to combat. The simplistic nature of SODIS is both its selling point and its downfall. There is a perception of SODIS as being a “poor mans” water treatment, i.e. only used by people who cannot afford a better option. In order for SODIS to lose the negative stigma, it needs to be “normalized”. This can only happen when people in communities see their neighbours using SODIS and thus it becomes the norm (Meierhofer & Landolt, 2009).

2.5 Light source

There are two categories for SODIS method experiments, those using actual sunlight and those using simulated sunlight. Running experiments using actual sunlight gives real world results. However, replication of the results may be difficult if the weather changes between experiments. These experiments also require the researcher to be located at the area of interest due to the change of solar irradiance with geographical location. Laboratory experiments on the SODIS method are able to control the conditions with more precision but have the disadvantage of trying to establish a correlation back to the geographical location of interest.

The sun produces light across a large spectrum, however the three main bands that are commonly referred to are ultraviolet (UV) light, visible light and infrared (IR) light. The wavelength range of UV light is from 100 – 400nm, 400 – 800nm for visible light and from 800 – 2450 nm for IR light as shown in Figure 2-3. The UV wavelength is often further divided into UV-C (100 – 280 nm), UV-B (280 – 315 nm) and UV-A (315 – 400 nm).

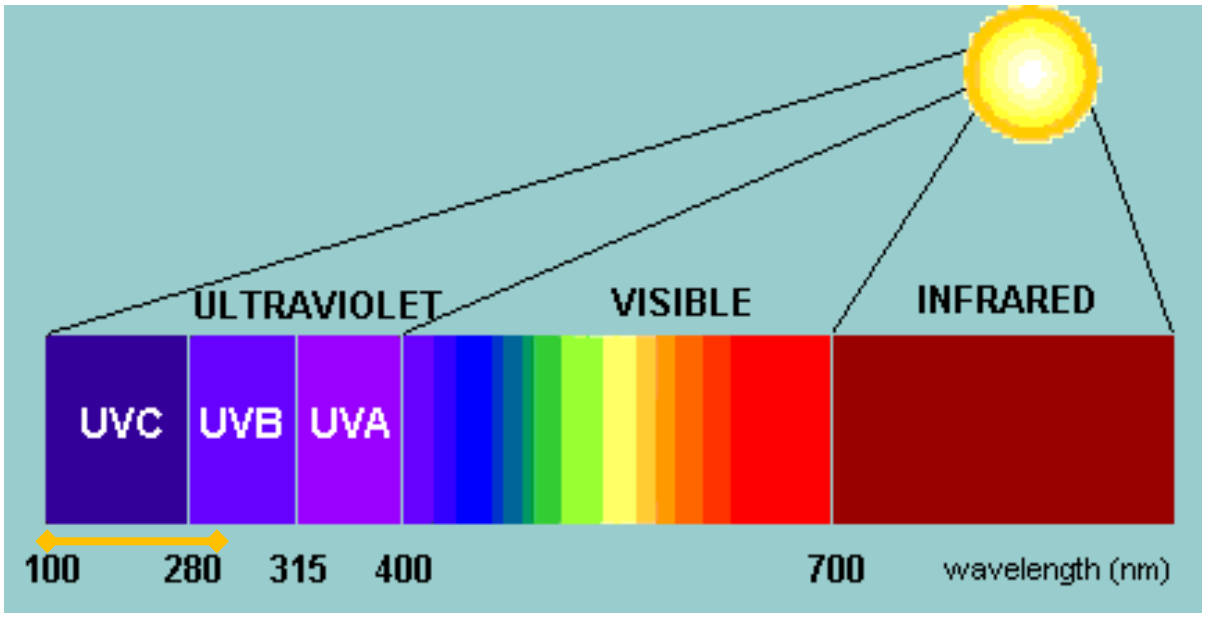

Figure 2-3: Solar Spectrum

Note: Adapted from https://www.quora.com/Why-would-an-open-box-solar-cooker-using-aluminium-foil-panels-produce-higher-temperatures-faster-than-an-open-box-solar-cooker-using-common-plane-mirror-panels

The orange line shown above the UV wavelengths in Figure 2-3 denotes the germicidal wavelength range of UV light being 100 – 290 nm. At these frequencies, the UV light has sufficient energy to cause direct inactivation of pathogens. Although the sun produces the full UV spectrum, the earth's atmosphere filters out all the UV-C and most of the UV-B wavelengths i.e. the germicidal wavelength range. Therefore, the majority of UV light that strikes the surface of the earth falls in the UV-A band. This UV-A light indirectly disinfects the water in the SODIS method and makes it safe for drinking.

2.5.1 Safety precautions when working with ultraviolet lights

Ultraviolet radiation has three distinct bands UV-A, UV-B and UV-C as previously illustrated in Figure 2-3. The shorter the wavelength the more energy that is contained, meaning that UV-C has more potential to do harm than UV-A. This is the reason why UV-C light is able to

inactivate pathogens directly and UV-A light has to do it indirectly. Pupils will not constrict in response to UV light by itself, therefore care needs to be taken to protect the eyes. Wearing UV glasses or using bright lighting with the UV lights will overcome this. Bright visible light constricts the pupils, reducing the amount of visible and UV light hitting the retina. Table 2-1 outlines some of the hazards that can occur with respect to the different frequencies of UV light.

Table 2-1: Ultraviolet Bands and the Hazards Associated with Them

Band	Wavelength	Primary Visual Hazard	Other Visual Hazards	Other Hazards
UV-A	315-400nm	Cataract of lens		Skin cancer, retinal burns
UV-B	280-315nm	Corneal injuries	Cataracts of lens, photokeratitis	Erythema, skin cancer
UV-C	100-280nm	Corneal injuries	photokeratitis	Erythema, skin cancer

Note: Retrieved from (Akram & Rubock, 2005). Reprinted with permission

Akram and Rubock (2005) from their studies in working with UV radiation have established the following limits:

1. For UV-A wavelength light of total irradiance 10W/m^2, the maximum unprotected exposure time should not exceed 1000s (16min 40s)
2. With the total radiant exposure not exceeding 104J/m^2 over the same time period.

2.6 Laboratory simulation of the SODIS method

When simulating the SODIS method in a laboratory the first question asked is, what light source is to be employed? Use of different light sources makes comparing results difficult, however Carratalà et al. (2016) recommend publishing results in terms of fluence rather than time to overcome this. Fluence is a measure of the irradiance that a body receives over a period expressed in units of kJ/cm^2. The three main light sources used in experiments with UV light are xenon arc lamps, metal halide lamps and UV fluorescent tubes. Both the xenon arc lamps

and metal halide lamps approximate the full solar spectrum i.e. produce UV, visible and IR light. UV fluorescent tubes only produce light in the UV bandwidth, which is what inactivates the pathogens during the SODIS method. Each type of light has its own advantages and disadvantages as discussed. High irradiance is not necessarily an advantage when simulating experiments. Bosshard, Berney, Scheifele, Weilenmann, and Egli (2009) found that with high UV intensities (>700 W/m^2) an overestimation of the results occurred due to light dose reciprocity failure. The reciprocity worked well for irradiance < 400 W/m^2, which is close to the intensity of natural sunlight.

Xenon Arc Lamps produce an intense full spectrum light by means of a plasma ball within a high-pressure xenon gas envelope. The plasma ball reaches temperatures of 6000-6500K at the cathode, which results in high heat output and often the need for water-cooling. Filters are necessary to eliminate the wavelengths not required. Due to the high pressure of the xenon gas within the lamp, there is a constant risk of explosion whether the lamp is on or not. This risk of explosion increases with the age of the lamp. Therefore the lamps need to be housed in an explosion proof casing and must be handled with care (Aphalo et al., 2012).

Metal Halide lamps emit a large number of individual spectrum that when combined produce light over a wide spectrum. The individual spectra are the product of the large number of different metals many of which are rare earth metals, interacting with the halogens to form halides. The difficulty lies in maintaining a controlled spectral output. This requires sophisticated power supply controls and well design lamp fixtures. These factors add significantly to the setup and operating costs (Aphalo et al., 2012).

The design of low-pressure mercury lamps or fluorescent tubes is such that they emit light over a specific spectrum. The lights work by passing an electric current through the mercury vapour, which becomes excited and produces UV radiation. A phosphorous coating on the inside of the tube converts this UV radiation into fluorescence. Using a lamp that only emits light in a desired spectrum does away with the need for filters and reduces the power consumption (Aphalo et al., 2012). Two commonly used fluorescent tubes are the UVB-313 and UVA-340. The name reflects the wavelength at the peak output of the lamp. UVB-313 has its peak output at a wavelength of 313 nm while the UVA-340 tube has its peak at a wavelength of 340nm. The two lights actually produce light in both the UV-A and UV-B bandwidths that can create confusion as shown in Figure 2-4. Accelerated ageing experiments require light with a shorter wavelength, like that emitted by the UVB-313. The UVA-340 light was designed in 1987 to

match the solar irradiance of the sun below 325nm so is used for investigating the effects of solar ageing in real time (McGreer, 2001).

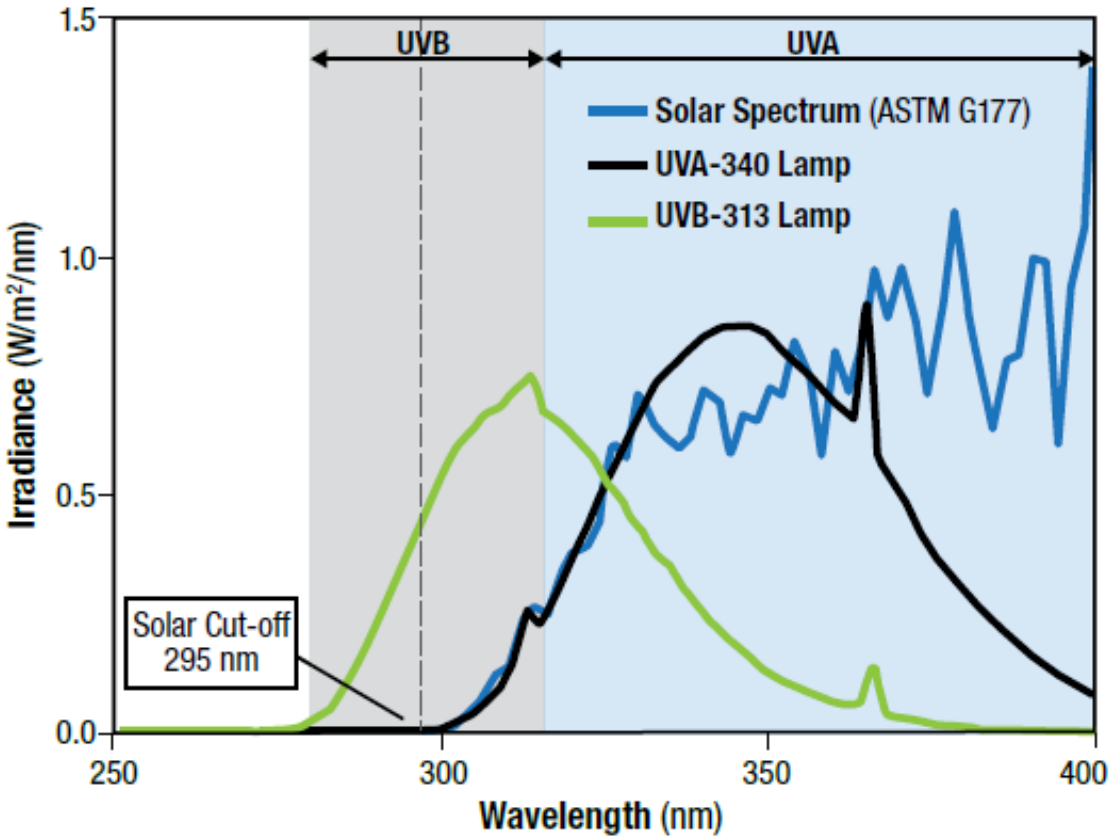

Figure 2-4: Comparison of UVA-340 and UVB-313 Lights with Solar Sprectrum. Copyright 2011 by (3M Weathering Resource Center) Reprinted with permission

The energy of the light with respect to frequency is shown in (1):

$$E = hf \tag{1}$$

Where E = energy of photons (J), h= Planck's constant (m^2kg/s), f = the frequency of light (Hz)

(2) shows the relationship between the frequency of light and the wavelength.

$$f = \frac{c}{\lambda} \tag{2}$$

Where c = speed of light (m/s), λ = wavelength of light (nm).

(3) is the energy of a photon in terms of its wavelength. It was obtained by substituting (2) into (1).

$$E = h\frac{c}{\lambda} \tag{3}$$

From (3) it can be seen that the energy of a photon is inversely proportional to wavelength of the light, i.e. the shorter the wavelength the more energy the light contains. The implication of this means that it is more important to match sunlight with the artificial light in the lower frequencies rather than the higher frequencies. The UVA-340 fluorescent tube as shown in Figure 2-4 is the preferred light source in ageing experiments as it closely approximates the solar light that reaches the surface of the earth at lower wavelengths. It does not emit visible or infrared light therefore does not heat the specimen under irradiation like a solar simulator. The drawback being that this results in only a loose correlation to actual sunlight ageing. It does however provide consistent comparison between tests with different variables (McGreer, 2001).

2.6.1 Ultraviolet light and its effect on plastic

When plastic bottles age there is a reduction in their transparency. This occurs either chemically as the plastic reacts with the UV light to form photoproducts, or mechanically due to scratches (Wegelin et al., 2001). The SODIS method relies heavily on the transmission of UV light through the bottle to inactivate the pathogens therefore it is important to maintain optimum transmission of UV light. Mechanical impediments such as scratches can be minimised through taking care of the bottles and not allowing them to slide on hard surfaces etc. Chemical degradation is harder to avoid as it occurs every time that the bottle is exposed to the UV light. The addition of ultraviolet stabilisers improves plastic bottles' resistance to sunlight. Over time however, photochemical reactions deplete these stabilising additives. The result of the depletion in stabilisers is a change in the chemical properties of the plastic and ultimately a reduction in the UV transmittance (Meierhofer & Wegelin, 2002).

Natural ageing of bottles is a diurnal process i.e. there are two parts. During the day, the plastic is exposed to solar radiation and heated; then at night, the plastic cools and contracts. McGreer (2001) listed continuous exposure to light and no temperature cycling as being reasons for having poor correlation between natural weathering and artificial weathering. One reason was that some chemical reactions need a rest period in order take place. Additionally the expansion and contraction of the plastic with changes in temperature creates physical changes in the plastic that do not occur under constant temperature. This is only applicable when trying to age bottles artificially rather than carrying out real time ageing.

2.7 Water source

2.7.1 Inter-source variation

Disinfection of drinking water using solar radiation or SODIS, was first studied by Professor Aftim Acra in 1984 (Luzi et al., 2016). Since then researchers have carried out numerous studies on the different aspects of SODIS. Recently Carratalà et al. (2016) discovered a significant difference in the performance of SODIS with different water sources. In their study Carratalà et al. (2016) used two water sources from Chennai, India and one water source from Lausanne, Switzerland. The Indian water sources were ground water and tap water whereas the Swiss water was tap water only. When comparing the inactivation of the MS2 virus, the results ranged from 6.7 log for Swiss tap water, 1.8 log for Indian tap water and 0.6 log for Indian ground water. From characterising the three waters, the high levels of iron and low organic matter in the Swiss water were postulated to be responsible for the high inactivation rate. The Indian tap water also had high levels of iron but it was surmised that any positive action from these ions was countered by high levels of organic matter also present. In their closing comments Carratalà et al. (2016) recommended that further research be used to confirm this. These results highlight the naivety of applying a "one size fits all" approach to SODIS. Not only do different locations receive varying amounts of UV radiation but also the characteristics of the source water at each location is likely very different. This stresses the importance of carrying out water characteristic testing prior to running site-specific experiments.

2.7.2 Conductivity

Due to seawater intrusion into the fresh water lens in Kiribati, the conductivity of the groundwater varies considerably depending on the time between rainfall events. White et al. (1999) found that the conductivity of the groundwater varied between 400 and 900 µS/cm. Rainfall patterns are responsible for the variation, with 400 µS/cm occurring after rainfall and 900 µS/cm after prolonged dry spells. Overall, the mean specific conductance was 700 µS/cm. Knowing that disinfection with the SODIS method is carried out indirectly by ROS, what effect if any would this change in conductivity have on the time required to disinfect the water?

2.7.3 Water pH

Chong et al. (2011) investigated the effect of pH with a TiO_2-K catalyst on the SODIS method. They found that as the pH decreased from 10.0 - 4.0 the inactivation rate increased. The pH, as the driver of the oxidation reaction was believed to be responsible as it can affect the surface charge of photo catalysts and photo oxidation rate (Chong et al., 2011). Fisher, Keenan, Nelson,

and Voelker (2008) also reported this same trend of increased inactivation with decreasing pH. In their experiments on citrus juice additives Fisher et al. (2008) believe that the increased inactivation was solely due to the low pH and not related to products in the citrus juices. The pH of the ground water in Kiribati is typically 8-8.3. South Tarawa in Kiribati is a coral atoll. When rainwater filters down through the coral sands into the groundwater lens, the coral or calcium carbonate ($CaCO_3$) increases the pH. The SODIS method and time required for disinfection was standardised based on considerable work carried out on large continents (Luzi et al., 2016). It is unlikely that these areas would have the same high $CaCO_3$ levels and high pH levels as Kiribati. To establish the applicability of the SODIS method and effect on time required for pathogen inactivation to Kiribati an investigation into the effect of this pH is required.

2.7.4 Water hardness

Water hardness is a measure of the total calcium and magnesium ions present in the solution. Tests for water hardness can be determined using Eriochrome Black T (ErioT) as an indicator in an ethylenediaminetetraacetic acid (EDTA) titration. ErioT turns from blue to pink by forming complexes with calcium and/or magnesium ions that are present as shown in (4). EDTA also forms metal complexes as shown in (5) and (6) with calcium and magnesium ions respectively. The ErioT complexes are less stable than the EDTA-metal complexes so will break down to supply metal ions to the EDTA.

$$Erio\ T\ (Blue) + Ca^{2+} = Erio\ T - Ca\ (pink) \quad (4)$$

$$Ca^{2+} + EDTA^{4-} = [Ca - EDTA]^{2-} \quad (5)$$

$$Mg^{2+} + EDTA^{4-} = [Mg - EDTA]^{2-} \quad (6)$$

Table 2-2 contains the results from testing carried out on Kiribati freshwater, Christchurch drinking water, the Okeover stream and seawater taken from New Brighton beach, both in Christchurch.

Table 2-2: Total Hardness Given in $CaCO_3$ for Water Samples from Kiribati, Christchurch Tap Water, Okeover Stream and New Brighton Beach

Water Source	Total Hardness (mg/L $CaCO_3$)
Kiribati (Fraser Thomas Partners, 2011)	116-630
Christchurch tap water	45
Okeover stream	74
New Brighton seawater	5252

Seawater from New Brighton had the highest total hardness in Table 2-2 with a value of 5252 mg/L $CaCO_3$. The sample from the Okeover stream and Christchurch tap water both has low water hardness. The high total water hardness baseline for Kiribati is the result of surface water percolating down through the $CaCO_3$ substrate. The spikes in hardness being an indication of saltwater intrusion into the lens.

2.7.5 Water turbidity

SODIS bottles require filling with the clearest water available. This is because turbid water prevents penetration of UV light and can shade pathogens from the irradiance. The three mechanisms for shading the pathogens are refraction, reflection and scattering (Crittenden, Trussell, Hand, Howe, & Tchobanoglous, 2012). To minimise the effects of shading an upper limit for turbidity has been recommended as 30 NTU (EAWAG, 1999). A rough test for this is the ability to read a newspaper headline under the bottle when looking vertically down through the bottle mouth of a 1.5L bottle (Luzi et al., 2016). For turbidity higher than 30 NTU, the water needs to be pre-treated with either filtering, settling or flocculation. If the turbidity is higher than 30 NTU and it is not possible to pre-treat the water to lower it, then another form of disinfection other than SODIS is to be used to ensure safe drinking water.

2.7.6 Water temperature

The SODIS method relies on UV light to disinfect the water the water through the production of ROS. Disinfection of the water is possible using thermal inactivation alone but the temperature needs to be greater than 55°C. (McGuigan, Joyce, Conroy, Gillespie, & Elmore-Meegan, 1998). Studies have shown that a synergistic effect between optical and thermal inactivation occurs when the water temperature rises above 45°C (McGuigan, Joyce, & Conroy, 1999). To test only the effect of optical inactivation without thermal inactivation, temperatures need to be kept below 42 °C (Kehoe, Barer, Devlin, & McGuigan, 2004)

2.8 Research aim and objectives

The SODIS method disinfects water and makes it safer to drink. It only has four steps and does not require extensive equipment. Variables that have an effect on the time required to inactivate the pathogens include pH and water depth. Additional variables that may affect the rate of pathogen inactivation include conductivity, total water hardness and bottle age. In increasing the rate of pathogen inactivation, a reduction in disinfection time occurs. With this reduced disinfection time, the SODIS method may become more attractive for people to use reducing the rate of child mortality. An increase in numbers of people using SODIS as an improved water source and reducing child mortality are both Millennium Development Goals that are of concern in Kiribati.

2.8.1 Aim

To investigate the performance of the SODIS method in Kiribati by simulating the conditions and water characteristics found there.

2.8.2 Objectives

1. A laboratory simulation of the SODIS method using the conditions typical of South Tarawa Kiribati will determine the die-off rates of pathogens due to different variables. Variables studied are:
    a. Conductivity
    b. pH
    c. Total water hardness
    d. Depth of water i.e. bottle size
    e. Age of bottle
2. The experimental results will be modelled and a comparison of inactivation coefficients and their statistical significance across all the variables investigated.
3. The applicability of the published SODIS method will be evaluated with respect to the time required for inactivation in Kiribati using the experimental results.

3 Methodology

There are two methods for carrying out SODIS experiments, either outside using natural sunlight or in a laboratory using an artificial light source. The experimental setup for this research used UV lights with indicator pathogen under controlled laboratory conditions.

3.1 Location of experiments

A temperature-controlled room within the Environmental laboratory at the University of Canterbury was utilized for running the experiments. This allowed the temperature and humidity to be fixed at 28°C and 50% respectively. These conditions were chosen to simulate the average conditions found year round in Kiribati (World Weather Online, 2016).

3.2 Ultraviolet lighting

3.2.1 Health and safety

Strict health and safety requirements were actioned while working with the UV lights. In particular with signage and personal protective equipment.

Signage

Working with UV light causes damage to the eyes and skin as outlined in Section 2.5.1. To address this hazard, signage was erected outside the temperature-controlled room where UV lights were operating which stated:

“Caution: High Intensity Ultraviolet Energy. Protect Skin and Eyes”

Personal protective equipment (PPE)

All people in the laboratory are required to wear a lab coat as standard procedure. To enter the temperature-controlled room while the UV lights were in operation additional PPE was required. In particular, a polycarbonate face shield was necessary as this protected the whole face not just the eyes. Due to its physical and chemical properties, polycarbonate blocks 99.9% of the UV wavelength light. Gloves were also required to be worn with no gap between them and the cuff of the lab coat.

3.2.2 Atlas ultraviolet lights

Atlas UVA-340 40W T12 fluorescent tubes were chosen as the UV light source due to their close approximation to the UV bandwidth of post atmospheric filtered sunlight. They also have a large irradiation field with allows for the use of full size SODIS bottles in the experiments. T12 fluorescent tubes represent old technology in terms of lighting due to the high levels of mercury inside them. For this reason, it is illegal to sell T12 tubes in New Zealand except for scientific research purposes. This made locally sourcing a 40W ballast very difficult. After much searching, the tubes were instead mounted to single 1200mm batons with 36 W Philips HF-P 136 TL-D III IDC electronic ballast. The batons were suspended in three sets of pairs from an overhead frame using chain so that their height could be varied as needed. Underneath the lights was a sheet of aged galvanised roofing iron. This was to simulate the common roofing material used in Kiribati (Stärz, 2015). The batons were spaced at 55mm centres centrally over a furrow in the corrugated iron. Dividers were placed in between each pair of lights to eliminate light interference from the adjacent lights as shown in Figure 3-1. The vertical clearance between the top surface of the bottle and the bottom of the fluorescent tubes was 24mm. The irradiance used for calculating the fluence was measured at the upper surface of the bottle.

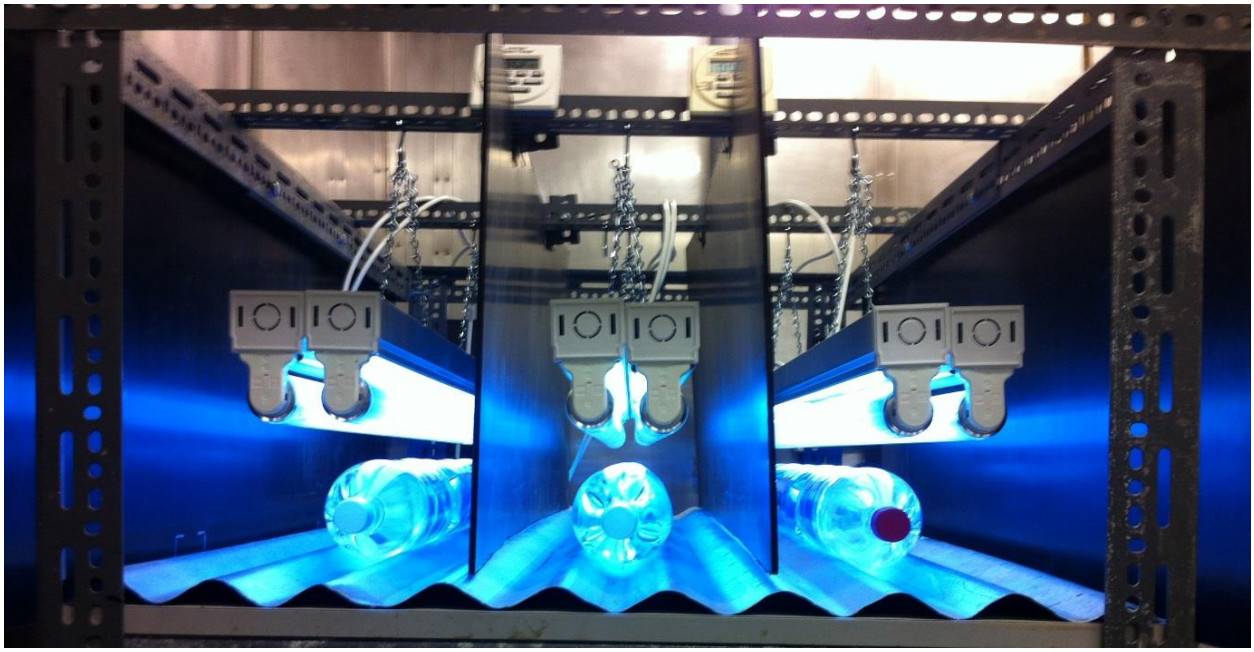

Figure 3-1: Arrangement of UVA-340 Tubes on Philips Batons Spaced at 55mm Centres over a Furrow in the Corrugation

3.2.3 Vertical irradiance

One drawback of carrying out laboratory experiments is that the light sources used to simulate sunlight are distance dependant. Light from the sun travels ~ 150 million km to reach earth so whether the bottle is located on the ground or on the laboratory roof, the irradiance it sees is essentially constant. However, this is not the case for the irradiance produced from a single UVA-340 tube as shown in Figure 3-2. The irradiance is highly dependent on the vertical separation between the bottle and light tube.

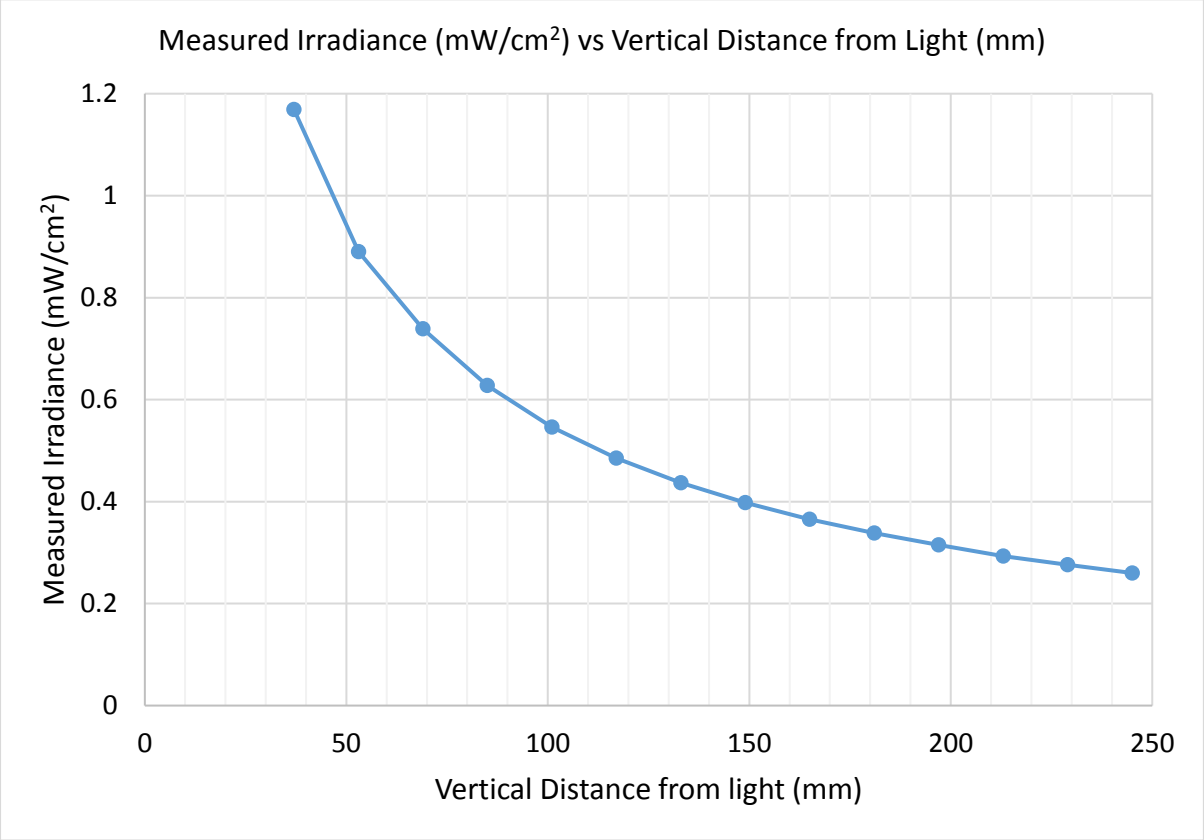

Figure 3-2: Irradiance of UVA 340 (mW/cm^2) Light with Respect to Vertical Distance (mm) for a Single Tube.

Figure 3-2 shows the measured irradiance on the y-axis, drops considerably for small changes in separation for vertical distances less than 100mm. Over 100mm in vertical distance, the drop in measured irradiation reduces before becoming approximately linear above 150mm.

3.2.4 Longitudinal irradiance

The Atlas UVA-340 fluorescent tubes are each 1200mm long; however, they do not emit light uniformly along their entire length. Figure 3-3 shows the measured irradiance (mW/cm^2) vs longitudinal length (m) for a single tube at a vertical distance of 120mm.

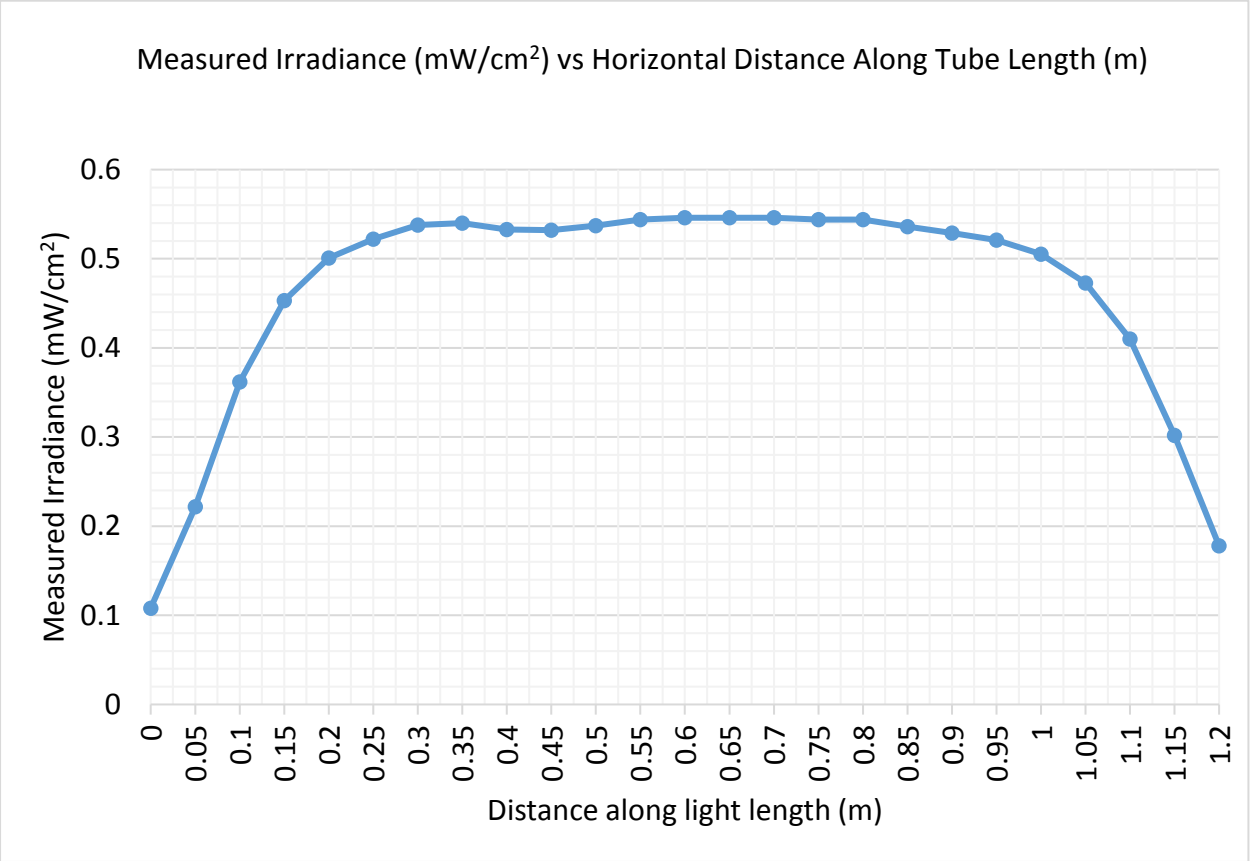

Figure 3-3: The Ultraviolet Irradiance Measured Along the Length of a Single Tube

The plot shows that the irradiance is approximately constant over the central region from 0.25 - 0.95m and then it drops off towards each end. The measured irradiance at 1.2m is higher than at 0m. This is likely due to the orientation of the UV sensor when recording the irradiance. To achieve consistent irradiation of the bottles and thus comparable results, the bottles need to be located in the central 0.7m uniform irradiance.

3.3 SODIS bottles

3.3.1 Bottle description

Commercially available 1.5L PET soft drink bottles were used for the SODIS experiments. The labels were removed from the bottles by soaking to minimise any scratching as shown in Figure 3-4. The bottles measured 305mm long, had a diameter of 93mm and an average mass of 40.83g.

Figure 3-4: Commercially Available PET Bottles Being Soaked to Remove the Labels

3.3.2 Bottle orientation

With each 1.5L bottle measuring 305mm in length, only two bottles / row can fit fully within the 700mm of constant irradiance delivered by the lights. By arranging the bottles as shown in Figure 3-5 i.e. with the narrower tops facing out, it reduced the depth of water that the light had to penetrate at the location where the irradiance was also decreasing. This bottle orientation was based on the research of Dessie et al. (2014), which showed that smaller diameters have higher inactivation rates than larger diameters. Therefore, nine bottles were irradiated with three bottles arranged under each pair of lights.

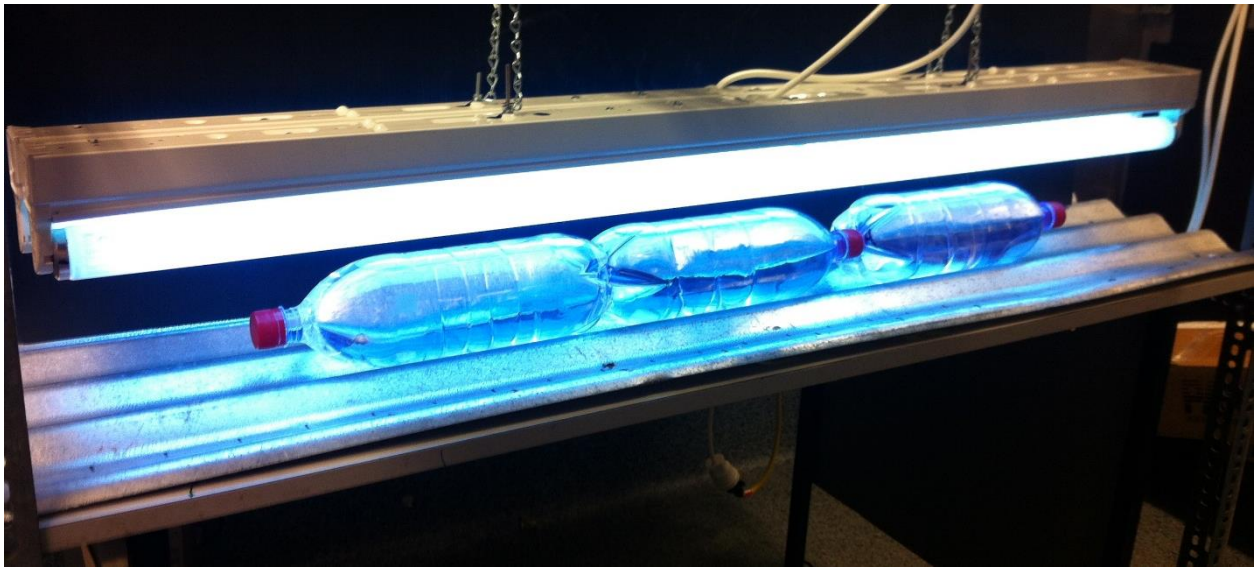

Figure 3-5: Photo Showing the Orientation of the Bottles with the Tops Facing Outwards

3.4 Sampling

3.4.1 Order of bottle removal

The bottles were arranged under the lights as set out in Figure 3-6. Every hour one bottle was removed and sampled according to the numbers shown. Following the sampling of the bottle, it was not returned to the experiment. This was repeated until after nine hours the final bottle had been removed and sampled. The experiments on each variable were all repeated a minimum of three times to allow for a triplicate of results. Rows 1, 2 and 3 as shown in Figure 3-6 each took turns at being sampled first, second or third. This was done to reduce errors due to small differences in irradiance produced by the different pairs of lights. Permanent identification was assigned to the bottles to allow a log of the hours each bottle spent under the lights to be kept. Based on this log the bottles were rotated to ensure all bottles received the same amount of hours under the lights.

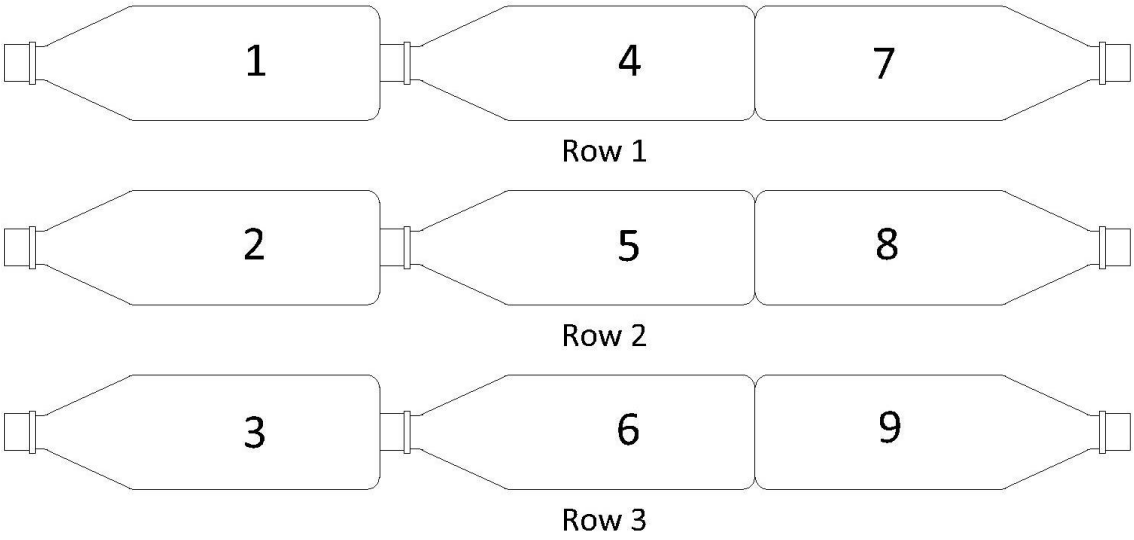

Figure 3-6: Order of Bottle Removal for Sampling

Following the removal of the first bottle from each row, the remaining two bottles in the row were longitudinally centred under the lights as shown in Figure 3-7.

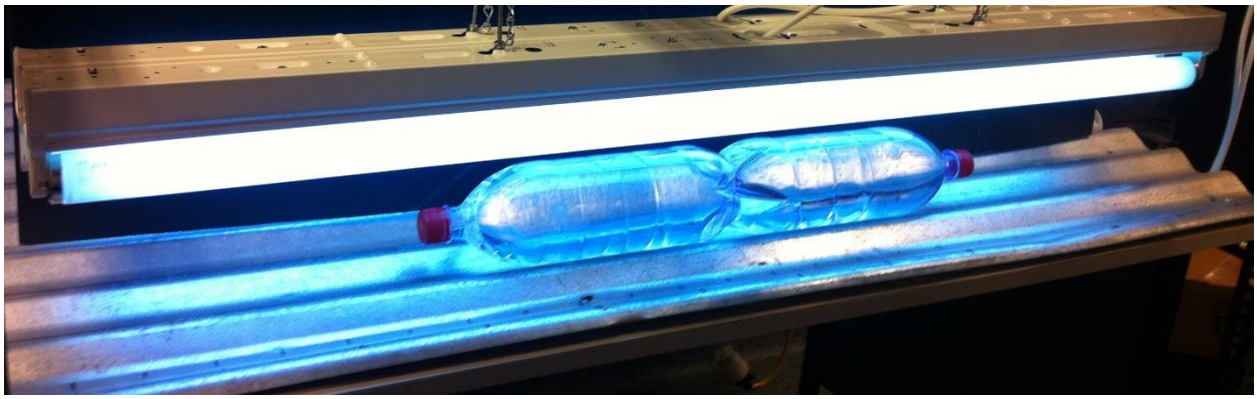

Figure 3-7: Two Bottles Longitudinally Centred Under the Light Following the Removal of the Third Bottle

3.4.2 Recording the temperature

A FLUKE 561 digital infrared thermometer was used to record the temperature of each bottle as it was removed from the experiment. This was to establish the cause of any inactivation, either optical only < 42°C, optical and thermal inactivation > 45°C <55°C, or predominantly thermal inactivation > 60°C. The temperature of the dark storage bottle adjacent to the experimental setup was also recorded. By recording the temperature of the dark storage bottle, it was possible to determine the temperature increase due to the ambient room temperature. With this increase known and the irradiated bottles temperature, the temperature increase due only to the UV light could be calculated.

3.4.3 Determination of pathogen numbers

Total coliform and *E.coli* were chosen as the indicator pathogens for the SODIS experiments. Testing for the presence of total coliforms and *E.coli* was accomplished with the Colilert-18 system produced by IDEXX. This system meets the requirements of the international standard ISO 9308-2:2012 for detecting both total coliforms and *E.coli*. It comes in single dose packaging and with the Quanti-tray/2000 can accurately establish Most Probable Number (MPN) from 1 - 2419 in a 100mL sample.

Colilert-18 has two main reagents, ortho-Nitrophenyl-β-galactoside (ONPG) and 4-methylumbelliferyl-β-D-galactopyranoside (4-MUG). During incubation, any total coliforms in the sample will use their β-galactosidase enzyme to hydrolyse ONPG. The two products produced are galactose and the yellow coloured ortho-nitrophenol. Therefore if the colourless sample solution changes to a yellow colour, this indicates a positive test for total coliforms (IDEXX laboratories, 2016).

In the test for the presence of *E.coli*, the β- glucoronidase enzyme of *E.coli* does the work. It cleaves the 4-MUG in the reagent producing 4-methylumbelliferone, which is a fluorescent compound. Figure 2-1 previously showed the relationship between total coliform and *E.coli*, meaning that a positive test for *E.coli* must also return a positive test for total coliforms. Thus, a yellow colour that glows under ultraviolet light is a positive test result for *E.coli*. By only targeting bacteria that have these two enzymes associated with them, it is possible to get accurate results even with samples that have high levels of suspended matter and heterotrophic bacteria. The Colilert 18 reagent can suppress samples that have up to 2 million heterotrophs per 100mL (IDEXX laboratories, 2016).

The samples from the experimental bottles were diluted to a volume of 100mL with de-ionised water (Millipore, Simplicity 185). This de-ioniser employed a germicidal lamp to ensure the de-ionised water was free from contamination. A Colilert 18 reagent (IDEXX) was mixed into the solution before sealing in a 97 well Quanti-tray/2000 (49 large wells and 48 small wells) with the Quanti-Tray Sealer PLUS (IDEXX). The trays were each incubated for 18 hours at 35°C ± 0.5°C in a Designer series incubator from Contherm. Following incubation, the total coliform MPN was determined by counting the number of large and small yellow wells and recording as XX/YY, where XX is the number of large wells returning a positive result and YY is the number of small wells returning a positive result. Using this number, the MPN is determined from the conversion sheet supplied by IDEXX in Appendix J.

MPN for *E.coli* was determined by counting the number of yellow wells that fluoresce using the Spectroline fluorescence analysis cabinet (Model CM-10A) and recorded as XX/YY. Figure 3-8 shows positive Quanti-tray/2000 results for total coliform and *E.coli* on the left and right respectively. Note that all fluorescent wells are also yellow, but not all yellow wells fluoresce.

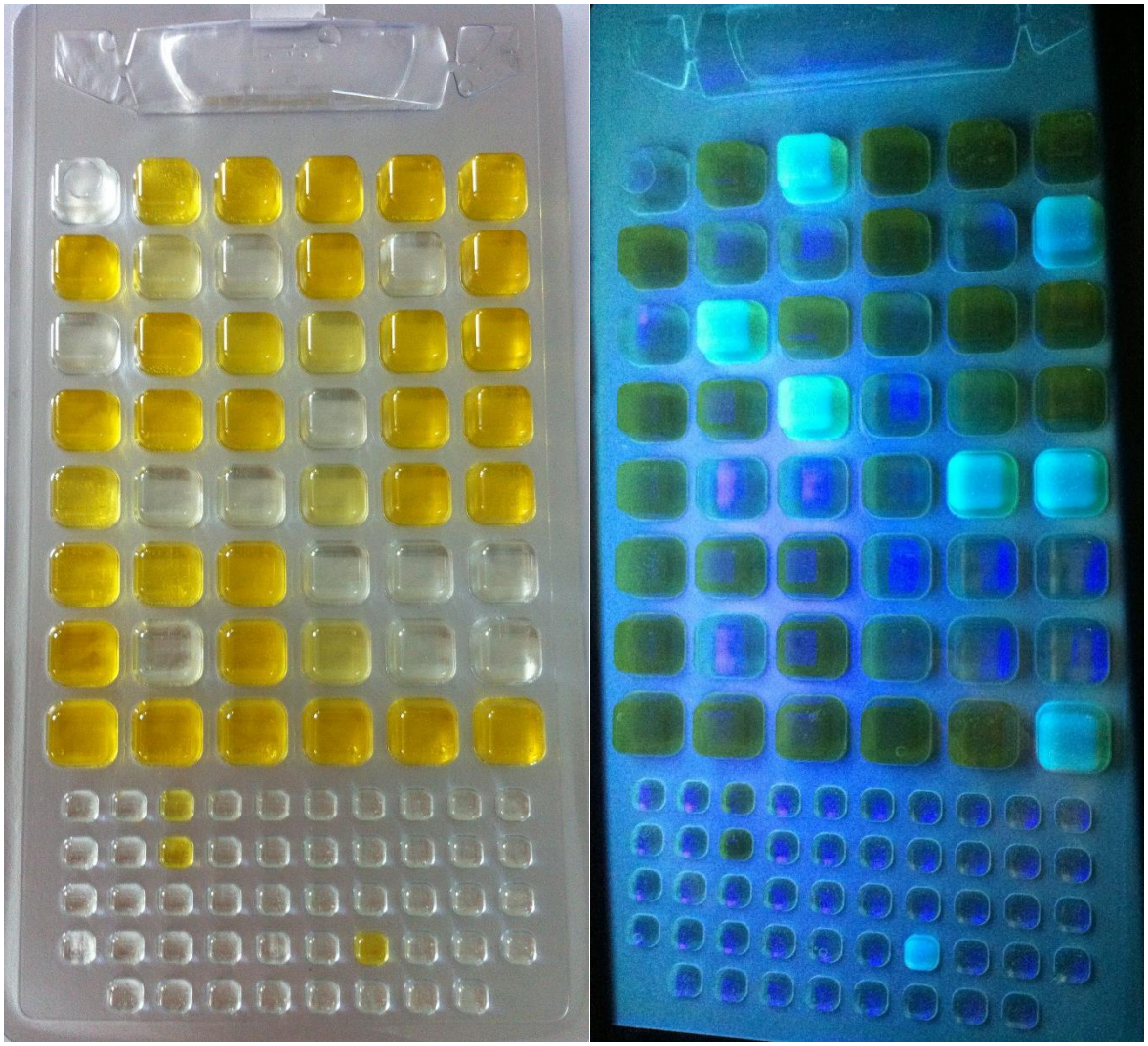

Figure 3-8: Left- Quanti-tray Showing 35/3 Positive Results for Total Coliforms (yellow wells), Right – The Same Tray Showing 7/1 Positive Results for E.coli (Fluorescent Wells)

3.4.4 Protocol for working with indicator pathogens

The source of the total coliform and *E.coli* for the experiments in this study was primary effluent. The implications of this is that numerous unknown pathogens within the effluent could also be incubated along with the known indicator pathogens. Adherence to the following protocol was essential for ensuring pathogenic safety:

1. Gloves and eye protection to worn at all times.
2. All glassware was sterilised in a Belimed Laboratory Steam Sterilizer, Model # LST 9-6-9 HS1 prior to use. The temperature of 121°C was maintained for 20 minutes at a pressure of 2200 mbar.
3. All glassware was thoroughly washed immediately following use prior to being sterilised.
4. All Quanti-trays were disposed of using the yellow biohazard waste bags regardless of their positive or negative results.
5. All surfaces were wiped down with ethanol, hot (70°C) water or suitable disinfectant.
6. Hands were washed thoroughly using soap after contact with Quanti-trays.

3.5 Conductivity experiments

3.5.1 Solution used

Following some adjustment of the sample dilutions and volumes of additives, the final recipe for the experimental water used in the conductivity experiments is shown in Table 3-1. Note that as there are three different conductivities being investigated, there are three different corresponding volumes of seawater being added.

Table 3-1: Volumes of Water Used for the Conductivity Experiments

Constituent	Volume (mL)	Conductivity (µS/cm)
Water – De-ionised	~ 20,000	~1
Seawater – New Brighton beach	120, 210, 285	52,000
Effluent – Christchurch wastewater treatment plant	100	
Solution 1	~20,000	400, 700, 900

3.6 Experiments with pH = 8.3

The initial conductivity experiments were carried out with pH= 6.5 – 6.8. To determine the effect of pH the same three conductivity experiments (400, 700 and 900 µS/cm) were repeated at a pH = 8.3. To achieve a pH of 8.3, a 10% solution of sodium bicarbonate (baking soda) was

added in 0.1mL increments to 1.5L of SODIS water. The solution was continuously mixed with an AGE magnetic stirrer (Velp® Scientifica) while the pH was being measured with a calibrated pH meter (EDT Instruments RE 357 Tx). After the pH had stabilised at 8.3, the final volume of baking soda was recorded as shown in Table 3-2. This amount of baking soda was then added to each of the experimental bottles.

Table 3-2: Volume of Sodium Bicarbonate Solution Added to Achieve a pH= 8.3 for Various Conductivities

Experimental Conductivity (µS/cm)	400	700	900
Volume (mL) of sodium bicarbonate added to achieve pH=8.3	0.8	1.1	1.2

3.7 Hardness experiments

The water initially used in the experiments had a total hardness of between 40 and 80 mg/L $CaCO_3$ depending on the conductivity. This is considerably lower than the ground water in Kiribati, which has a total hardness of typically 500-600 mg/L $CaCO_3$. To increase the hardness of the water, calcium chloride was added. Calcium chloride ($CaCl_2$) is a very soluble white powder that was dissolved in de-ionised water to make a 40% solution. Following the mixing of the SODIS water and decanting into the experimental bottles, each bottle was dosed with 3mL $CaCl_2$ 40% solution prior to the pH being adjusted to 8.3. This achieved a total hardness of approximately 540mg/L $CaCO_3$.

3.7.1 Hardness testing

The total hardness was confirmed with the following method prior to the irradiation being carried out.

- Measure 25mL of sample into a white porcelain dish
- Add 25mL of de-ionised water
- Add 1mL of ammonia buffer (Buffer solution shown in Table A-1)
- Add 1-2 drops of the indicator solution (Indicator solution shown in Table A-1)
- Titrate with EDTA to a blue endpoint
- Report the amount of hardness present per litre of sample, knowing that 1mL of EDTA added = 40mg/L of Hardness as $CaCO_3$.

With the addition of $CaCl_2$, the conductivity was also inadvertently affected. It was decided to continue with elevated conductivity even though it would not be directly comparable to the previous experiments. The typical characteristics of the hardened SODIS water used are shown in Table 3-3.

Table 3-3: Typical Characteristics of SODIS water for Hard Water Investigation after Adding Calcium Chloride.

Water Characteristics	pH	Conductivity (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Total Dissolved Solids (mg/L)
Typical values	8.3	1830	540	1188

3.8 Water depth

To determine the effect of water depth on the inactivation of the pathogens, the 1.5L 93mm diameter bottles were replaced with 355mL bottles with a diameter of 60mm. The same hard water solution from the hardness experiments was replicated to provide direct comparison.

The initial experimental water solution volume was reduced from 20,000mL to 4500mL to match the reduction in volume of the bottles. This resulted in a similar reduction of seawater and effluent being added to achieve the same conductivity and contamination levels. The bottles were arranged into two rows, with five bottles in one row and four bottles in the other. Once again the tops of the bottles were orientated to face outwards as shown in Figure 3-9.

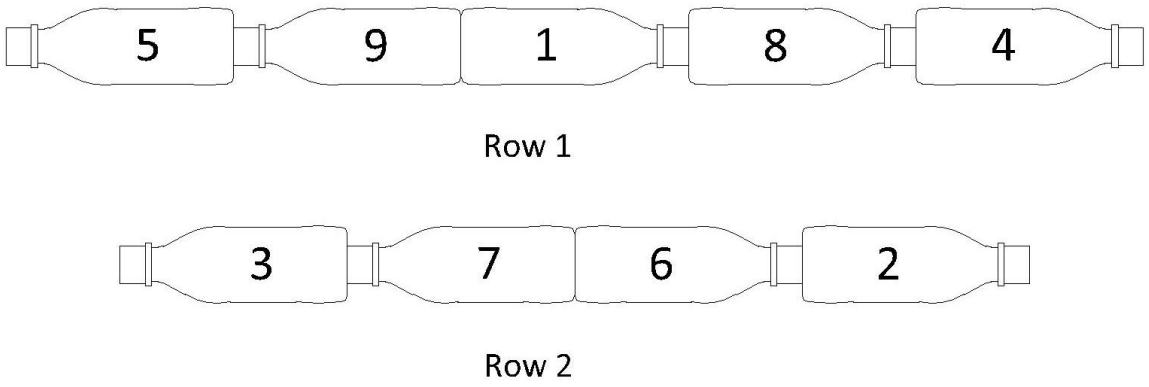

Figure 3-9: Order of Small Bottle Removal

The bottles were removed and sampled one every hour in the numbered sequence shown in Figure 3-9. Note, after bottle 1 was removed the remaining bottles in Row 1 were centralized as shown in Figure 3-10.

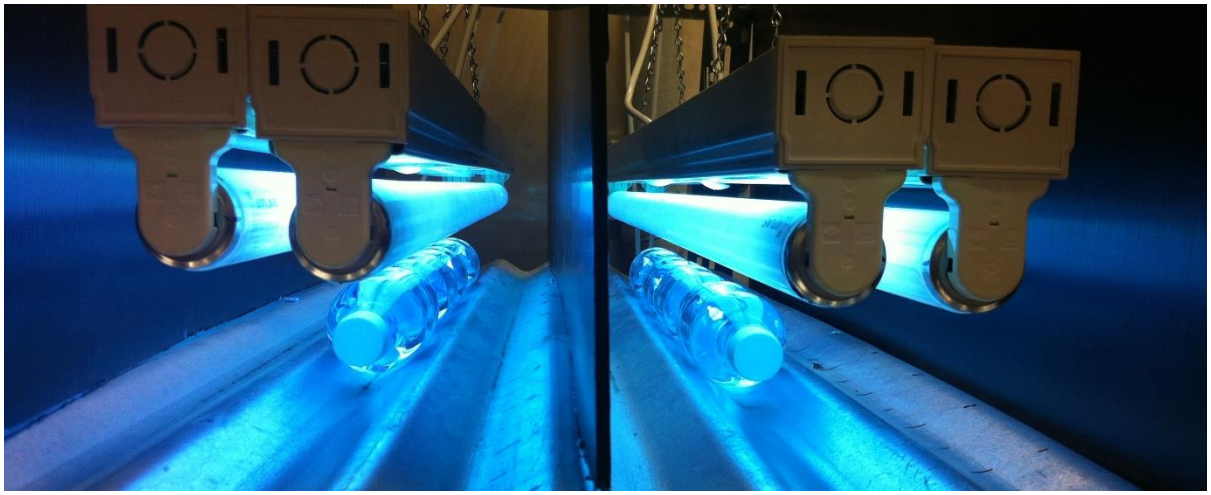

Figure 3-10: Irradiation of Small (355mL) Bottles

The fluorescent lamps were lowered to maintain the same bottle / light separation distance of 24mm that was used for the 1.5L bottles. This was to ensure the same irradiance on the top surface of the smaller bottles that was received by the larger 1.5L bottles.

3.9 The effect of ultraviolet light on PET

3.9.1 Ageing PET bottles using ultraviolet light

To investigate the effect of ageing due to UV irradiation on PET bottles, 3 sets of PET bottles were filled with Christchurch tap water and artificially aged under UV lights. The bottle characteristics are listed in Table 3-4.

Table 3-4: Characteristics of Aged Bottles

Quantity	Material	Height (mm)	Diameter (mm)	Volume (L)	Mass (g)
9	PET	305	93	1.5	40.8

The bottles were arranged under the lights in a 3x3 matrix as per Figure 3-6 with an average irradiance measured at the top surface of 2.4 mW/cm^2. Every 12 hours the bottles were rotated ¼ turn and shuffled across one position, i.e. the left hand bottles were moved to the centre, the centre bottles were moved to the right hand side and the right hand bottles were moved to the left hand side. This was to ensure even weathering of the bottle circumference and to eliminate any effects from the lighting irradiation differences. To account for diurnal effects, every two days the bottles were removed from the lights and placed in dark storage at 4°C for 10 hours. After ageing for a total of 1000hrs, the first set of three bottles was removed from the lights and kept in dark storage. This was repeated for the second set of bottles following another 500 hrs for a total of 1500 hrs irradiance. The final set of three bottles was aged under the lights for a total of 3000hrs.

3.9.2 Measuring the effect of artificial ageing on PET bottles

To measure the effect of UV ageing on the PET plastic, the transmission of UV light was recorded for all the aged samples and compared to a young sample that had been aged for 150hrs. The transmission of UV through the PET was measured with an Illuminance UV Recorder (TR-74U*i*, T and D). This was secured midway under a single UVA340 light with non-reflective "book ends" secured 60mm either side of the sensor for a total gap of 120mm. The setup used to obtain the baseline irradiance is shown in Figure 3-11.

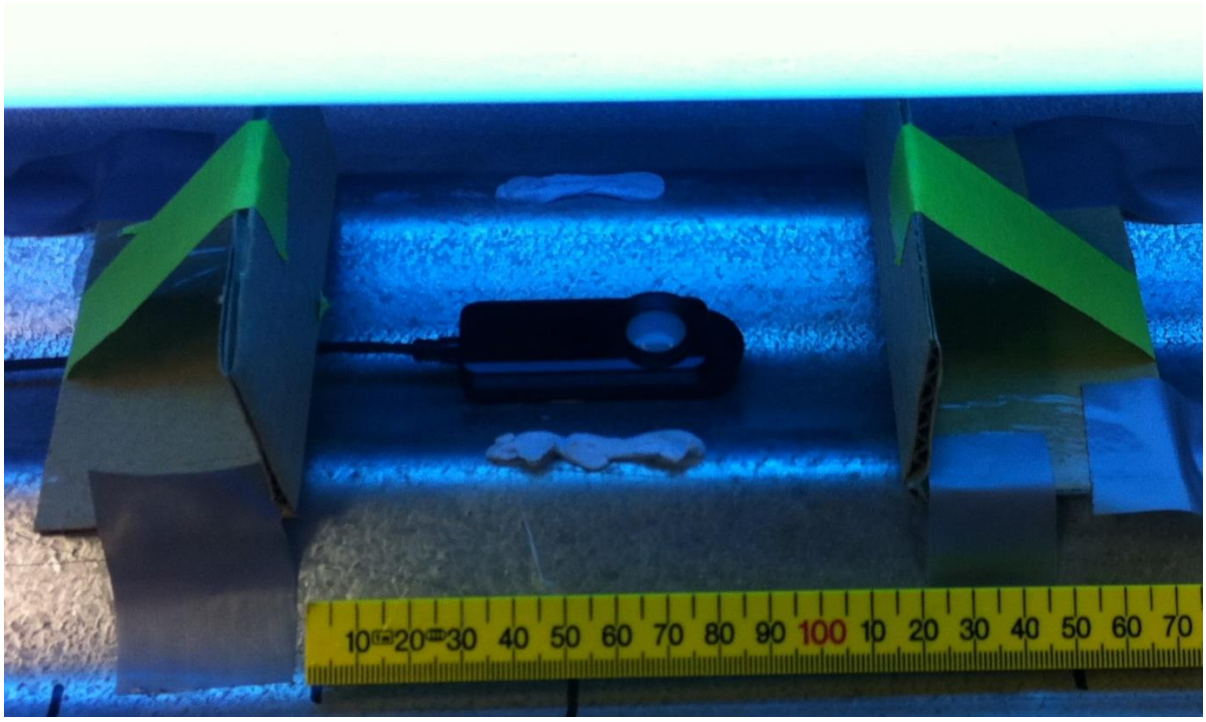

Figure 3-11: Experimental Setup for Recording the baseline UV Light Transmission.

One ring was cut from in between the stiffeners around the centre of each aged bottle. The ring was then cut into two samples that were standardised at 145mm x 40mm, which was half the perimeter of the bottle as shown in Figure 3-12.

Figure 3-12: Location of Where PET Samples are Removed From 1.5L bottle

Following the baseline recording, samples were placed centrally over the UV sensor and secured taut between the crests of the corrugated iron as shown in Figure 3-13. It should be noted that the probe has a black UV sensor, which is centrally located, and a white illumination sensor, which is offset. It was the black sensor that the samples were centrally located over in Figure 3-13.

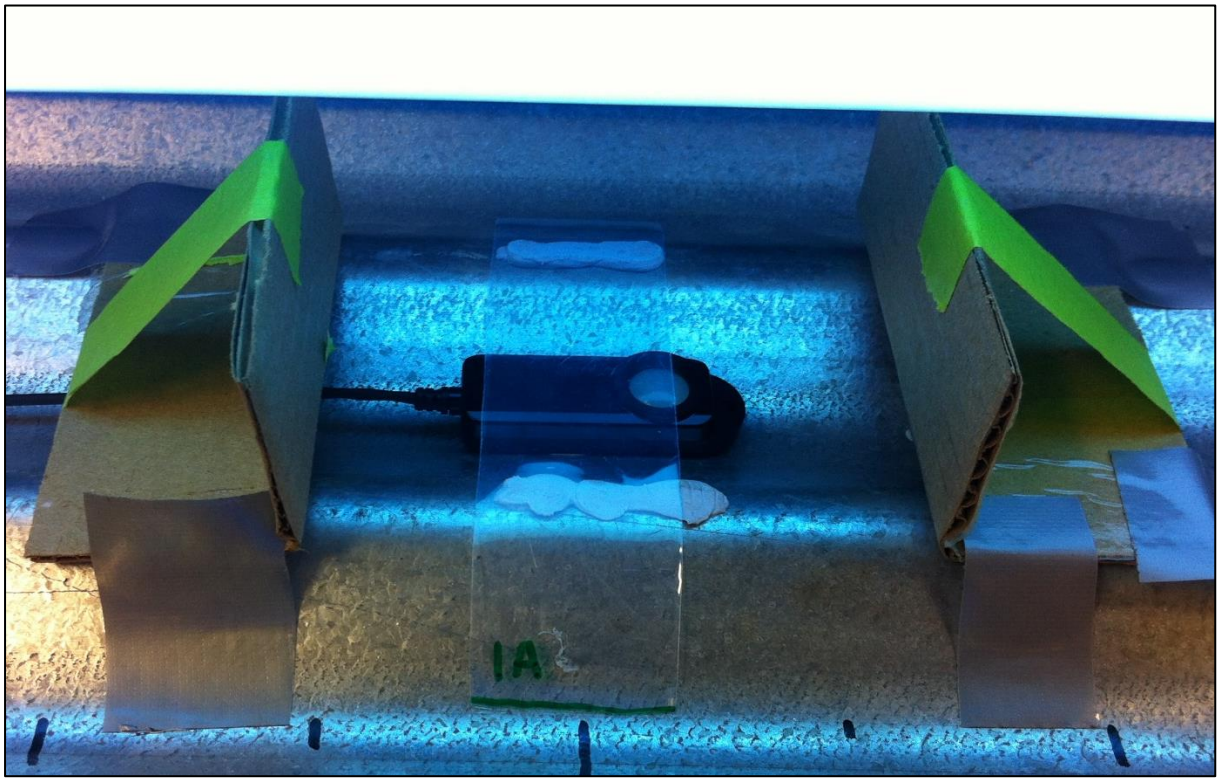

Figure 3-13: Sample from a 1.5L PET Bottle Being Tested for UV Transmission

The recording of the irradiance was delayed for 2 minutes after fitting the sample to allow the meter to stabilise and the sample to warm up. The purpose of the "book ends" was to stop any light from bypassing the PET sample and going straight to the UV sensor. A total of 24 samples were tested, these being six samples from each of the experiment bottles (150hr), the 1000hr bottles, the 1500hr bottles and the 3000hr bottles.

3.10 Reporting results

3.10.1 Plotting results

Plots provide a clear and easy way of presenting SODIS experimental results in a visual way. They are useful in spotting trends that have occurred as the experiment progressed. Most commonly, the y-axis is a Log_{10} of the pathogen population (C) divided by the initial pathogen population (C_0). The negative values indicate that a reduction in the total coliform population is occurring as the experiment progresses. A -1.0 Log_{10} value = 90% reduction, -2.0 Log_{10} = 99% reduction, -3.0 Log_{10} = 99.9% reduction etc. An example of this is shown in Figure 3-14.

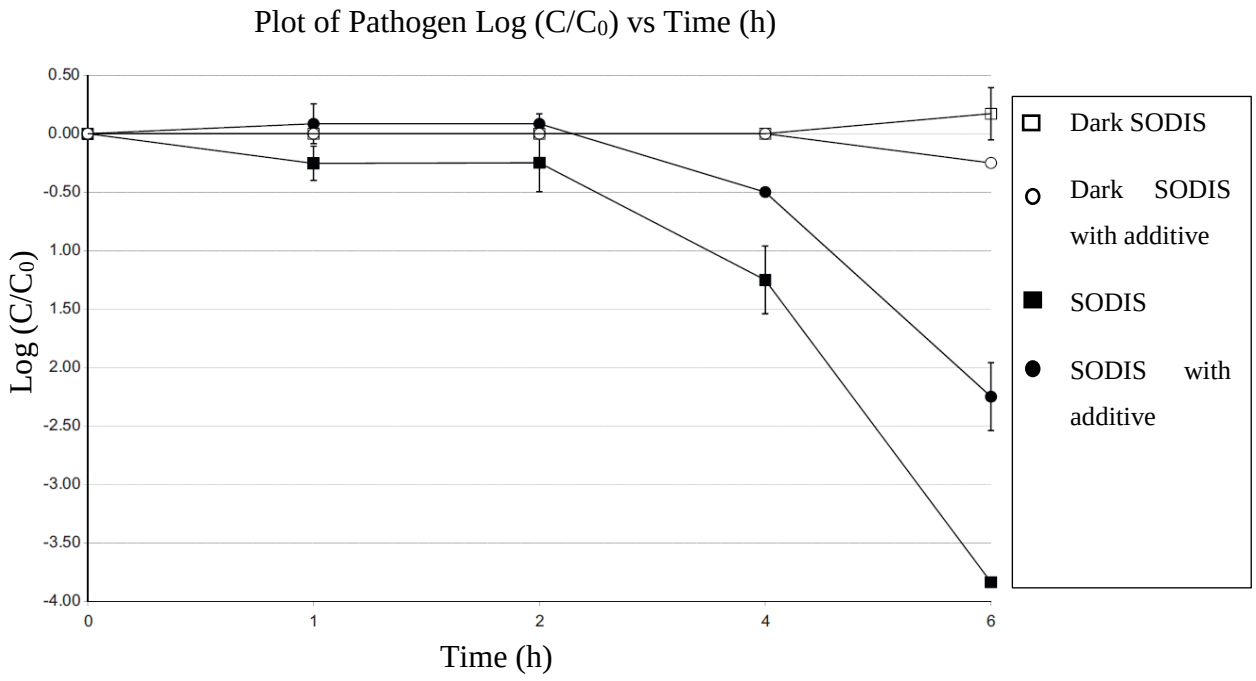

Figure 3-14: Example Plot of Pathogen Inactivation (log(C/C₀)) vs Time (h)

Note: Adapted from (Heaselgrave & Kilvington, 2011) p.4. Reprinted with permission

Using time of the x-axis like Figure 3-14 is easy to understand, but makes comparison between experiments using different light sources or geographical locations difficult. For this reason, fluence with units J/cm^2 or kJ/m^2 is commonly used. Fluence is a term for the cumulative radiation dose or intensity of light received multiplied by the time it took to receive it. As fluence uses information about both time and irradiation it makes comparing results between experiments easier.

3.10.2 Modelling results

Linear

Modelling of the plotted data allows for comparison between experiments. As the comparison is carried out between the models and not the raw data, it is important that the model is an accurate representation of the results. There are several models that can be used with the simplest being the linear model. (7) gives a linear approximation of the results data for a SODIS experiment.

$$Log_{10}\left(\frac{C}{C_0}\right) = -k_1 F + k_2 \quad (7)$$

Where F = fluence (J/cm^2), k_1 = slope (cm^2/J) and k_2 = y-axis intercept.

The advantage of using a linear approximation is that it is quick to fit over models that are more complicated. The disadvantage is that it fails to account for non-linear changes in the data; i.e. lags and tails. Lags occur at the start of an experiment and are identified as a flat spot on the graph as seen in the first two hours of Figure 3-14. This represents no significant change in pathogen population for a change in time or fluence. Tails occur towards the end of an experiment where again for increasing time or fluence there is little or no corresponding change in deactivation. This would appear as a plateauing of the data. The fit of the line to the data is checked using the least squares approach or the Pearson correlation coefficient values (R^2). R^2 values range from 0.0 being a very poor fit to 1.0, which would be a perfect fit of the line to the data. It should be noted that a high R^2 value does not necessarily mean that the linear line is appropriate; however, this can be checked by plotting the residuals. The residuals plot is a graphical representation of the differences between the modelled results and the actual results. As the differences can be positive or negative, the residuals should look like randomly scattered points straddling a centre-line. If there is a clear trend to the scatter, then it is likely that the linear line is inappropriate.

Advanced

To model the lag, linear and tail components that can occur in the results accurately, Chong et al. (2011) investigated fitting different models to their data as shown in Figure 3-15. The three models explored were the Hom model, the modified Hom model and the Hom-Power model. The models have increasing complexity with two, three and four parameters respectively.

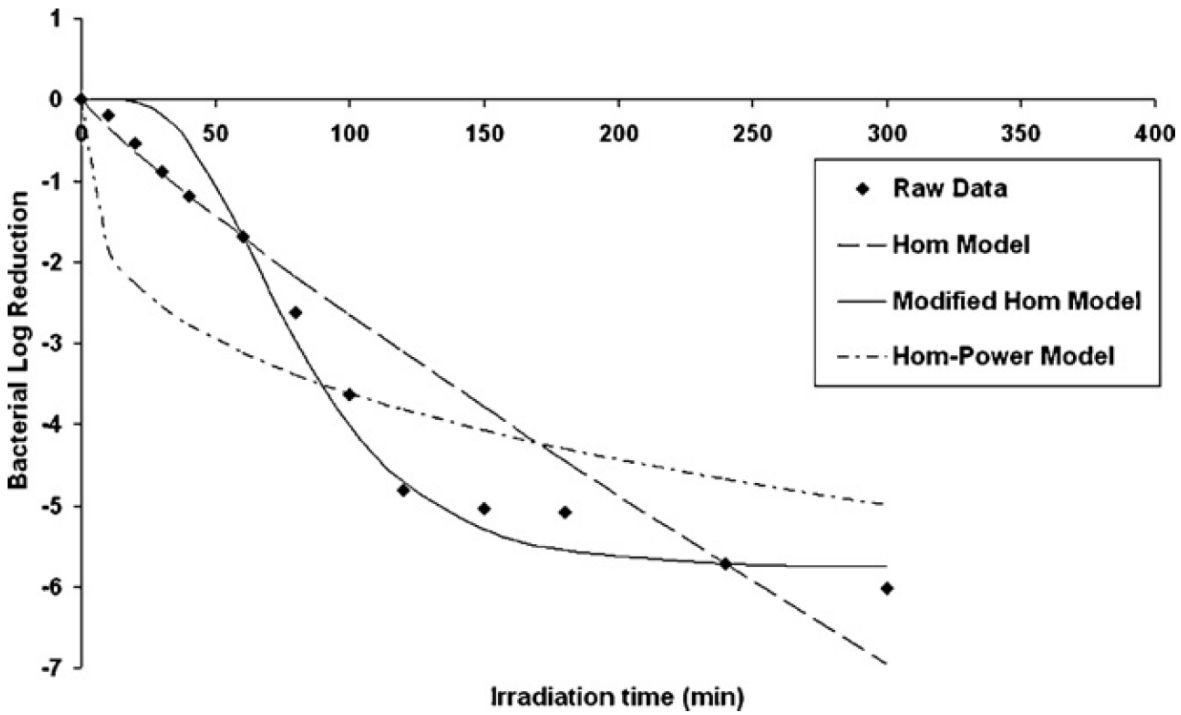

Figure 3-15: Comparison of Different Models Fitted to Data. Copyright by Chong et al. (2011). Reprinted with permission.

The modified Hom model approximates the data very well in Figure 3-15. The drawback in using models that are more complicated is that you need sufficient raw data points to obtain stable solutions. Therefore, any increased accuracy obtained by using a more complicated model can be lost due to model instability when data is limited. This is often the case for science experiments investigating many variables within constrained timeframes and budgets.

Lag corrected

With the SODIS method, it is not necessary to model all the data from an experiment, as it is only the final inactivation rate that is of concern. Fisher et al. (2008) used linear models for all their experiments that investigated the effects of pH, temperature, hydrogen peroxide and copper on the SODIS method. For data with obvious lags, they omitted the lag period and only modelled the linear data. Their justification was that most of the data was well represented by linear modelling ($R^2 > 0.9$) and that removing the lag data would not change their overall conclusions. Removing the lag data also improves the linear fit of the line and thus the accuracy.

Skew

The skew tests the experimental values against the mean value to determine the skewness. It can be used to establish if the values are normally distributed. If skew = 0, the data is symmetrical. A positive skew indicates the data skews to higher values, with the reciprocal being the case for negative skew. The skew of the results is determined using (8).

$$skew = \frac{n}{(n-1)(n-2)} \sum \left(\frac{x_{i-\overline{x}}}{s}\right)^3 \quad (8)$$

Where n = number of entries, x_i = value of entry i, $\overline{x}$ = entry mean, s = standard deviation

If the data is determined to be significantly skewed i.e. skew $> 2\sqrt{\frac{6}{n}}$, then the data is said to be NOT normally distributed.

T Test

T tests are used to determine whether the difference between two sets of numbers is due to variability within the set or actually, because the sets are different. Other methods also do this however, the T test works well for small sample sizes (n < 30). A value of p is assigned, often p = 0.05 is be used as a benchmark for statistical significance. Therefore, if the T test result is less than 0.05 that equates to less than 5% probability that the values are the same, or simply put "the difference in values is statistically significant for p=0.05". When using the T test there are three options: paired test, unpaired equal variance and unpaired unequal variance. Paired tests are used for dependant variables e.g. a SODIS experiment was carried out by sampling the same bottle every hour. For SODIS experiments that sample each bottle only once i.e. independent results unpaired T tests are used. To determine whether the variances are equal or unequal an F test needs to be carried out.

F Test

F tests determine the probability that variances are not different. A benchmark of 0.05 is often used. This means that there is a 95 percent chance the variance are equal. Therefore, for an F test result > 0.05 the unpaired equal variance T test would be used. For results from the F test < 0.05 an unpaired unequal T test would be used.

3.10.3 SODIS experiments

The raw data from each SODIS experiment was plotted Log(C/C_0) against fluence (J/cm^2), where C = the population of the pathogen and C_0 = the initial pathogen population. A visual check for lag was carried out and data points demonstrating lag were removed before modelling. This was done as only the final result and trend of the experiment was of interest.

A linear approximation was used to determine the slope and intercept of the line for each of the three experiments carried out using (7). Having 10 data points allowed for good linear approximation but was insufficient to obtain stable results for more complicated models e.g. the modified Hom model. The mean slope was calculated from the three experiments carried out on each variable. Knowing the mean slope, the skew was determined using (8). The T test was carried out using the built in T.TEST() function in EXCEL 2013 with p = 0.05 used as a benchmark for statistical significance. The variances of the slope values were checked using the F.TEST() function in EXCEL 2013. The result determined whether the unpaired T test carried out was for equal or unequal variances.

3.10.4 Aged bottle tests

Using the irradiance data gathered from the aged PET bottles tests, the mean from each set of bottles was calculated. These results were checked for significant skew before carrying out a T test and an F test using the built in EXCEL 2013 functions T.TEST() and F.TEST(). The results were plotted in a box and whisker graph with the statistical significance entered into a table to allow for comparison between different age bottles.

4 Results and discussion

4.1 Preliminary results

Typical water parameters

Water samples collected from the Okeover Stream at the University of Canterbury and New Brighton beach Christchurch were tested to determine the local water characteristics. The results are listed in Table 4-1 with results from testing carried out by Fraser Thomas Partners (2011) on Kiribati groundwater.

Table 4-1: Water Characteristics for Three Different Water Samples

Water Source	pH	Specific Conductance (SC) (µS/cm)	Total Hardness (mg/L $CaCO_3$)
Kiribati	8 - 8.3	400 - 900 (mean =700)	116-630
Okeover stream	~7.3	163	74
New Brighton seawater	~8	52,000	5252

The pH of the Okeover stream water was the lowest of the three locations tested with pH ~ 7.3, the other two sources being around 8.0. The SC varied considerably between the three sources with seawater being orders of magnitude higher than the other two. The elevated values of SC obtained from Kiribati indicated a possible intrusion of seawater into the freshwater lens. This was further reinforced with the 900 µS/cm reading being obtained after prolonged dry spells. The total hardness of seawater was the highest, followed by Kiribati ground water and finally Okeover stream water. Kiribati is a coral atoll so the water absorbs calcium ions as it passes through the coral sand into the freshwater lens. The fluctuations in hardness further illustrate the intrusion of seawater into the fresh water lens.

4.1.1 Pilot experiment 1

The first pilot experiment was designed to check the pathogenic inactivation of raw water with a SC of 400µS/cm. Solution 1 was prepared by combining 70mL of seawater with 14,930mL of water from the Okeover stream as per Table 4-2 below.

Table 4-2: Volumes of Different Waters to Make Initial Conductivity Solution

Constituent	Volume (mL)	Specific Conductance (µS/cm)
Water – Okeover Stream Christchurch	14,930	163
Seawater – New Brighton beach	70	52,000
Solution 1	15,000	484

Primary effluent from the Christchurch wastewater treatment plant and water from the Okeover stream were tested for total coliform and *E.coli* utilising the Quanti-tray system by IDEXX. Knowing the initial pathogen concentration, a suitable dilution for the experiment was calculated as shown in Table 4-3.

Table 4-3: Volume of Primary Effluent Added to Solution 1 to Obtain Approximate Starting Dose of E.coli

	Volume (mL)	Estimated E.coli (MPN)
Primary effluent	60	4,250,000
Solution 1	14,940	12
Final SODIS solution	**15,000**	**18,407**
Dilution of Primary effluent	**250**	

The mixed solution from Table 4-3 was decanted into the 9 x 1.5L PET experiment bottles. These were arranged in three rows of three under the UVA-340 fluorescent tubes as per Figure 3-6. From the remaining mixed solution, a sample was taken to establish an initial MPN. This was recorded as bottle 0 along with the results for the other bottles in Table 4-4.

The Quanti-tray dilutions were based on best estimates of how susceptible the pathogen would be to the UVA-340 irradiation. Table 4-4 shows the results for the first pilot experiment. In column 1 is the bottle number and time from the start of the experiment. Column 2 contains the dilution of the sample in the Quanti-tray. The number of positive large wells / small wells are displayed in column 3. For total coliform, this was the number of yellow wells and for

E.coli, it was the number of yellow wells that fluoresced under UV light. Column 5 is the product of column 2 multiplied by column 4. Columns 6 – 8 are the results for *E.coli*. Positive results for total coliforms were registered for hours 1 and 2 then not again until hour 9 as seen in column 3. For *E.coli*, positive results in column 6 were only recorded for the first hour. The lack of positive results does not mean that there are no pathogens, instead it means that the MPN in the tray is <1.

Table 4-4: MPN Results for Pilot Experiment 1

Col 1	Col 2	Col 3	Col 4	Col 5		Col 6	Col 7	Col 8	
Bottle	Dilution	Total coliform	MPN	Total coliform MPN		E.coli	MPN	E.coli MPN	
0	**2500**	**19/2**	**25.9**	=	64750	**1/1**	**2**	=	5000
1	2000	9/0	9.8	=	19600	5/0	5.2	=	10400
2	1250	1/0	1	=	1250	0/0	<1	<	1250
3	500	0/0	<1	<	500	0/0	<1	<	500
4	250	0/0	<1	<	250	0/0	<1	<	250
5	100	0/0	<1	<	100	0/0	<1	<	100
6	50	0/0	<1	<	50	0/0	<1	<	50
7	25	0/0	<1	<	25	0/0	<1	<	25
8	10	0/0	<1	<	10	0/0	<1	<	10
9	2.5	1/0	1	=	2.5	0/0	<1	<	2.5

Improvements from Pilot experiment 1

The first pilot experiment was a success in that it demonstrated a reduction in the MPN of total coliform and *E.coli* in the SODIS water from the UV irradiation. Three improvements came from this experiment:

1. Revision of the Quanti-tray dilutions to ensure more positive readings and therefore establish an actual MPN
2. A control bottle to be kept in the temperature-controlled room shielded from the UV lights to establish whether the pathogen reduction is from the UV light or temperature.
3. The initial solution volume to be increased to ~ 20,000mL and seawater added until the desired conductivity is achieved. The effluent dilution is not critical as the solution is sampled to establish the initial MPN of total coliform and *E.coli* before commencing the experiment.

4.1.2 Pilot experiment 2

The improvements listed in 4.1.1 were implemented into the second experiment. The SODIS water (solution 2) was mixed according to Table 4-5.

Table 4-5: Volumes of Water Used for Pilot Experiment 2

Constituent	Volume (mL)	Conductivity (µS/cm)
Water – Okeover Stream Christchurch	~ 20,000	163
Seawater – New Brighton beach	85	52,000
Solution 2	~20,000	404
Effluent – Christchurch wastewater treatment plant	80	

The mixed solution was decanted into nine 1.5L experiment bottles and one dark control bottle. The dark control bottle was kept in the same temperature controlled room but shielded from the ultraviolet light for the duration of the experiment before being sampled. The purpose was to determine whether the pathogen inactivation was due to the temperature or the radiation emitted by the UV light. After the nine experiment bottles had been arranged under the UV lights in the 3x3 matrix as per Figure 3-6, the remaining solution was sampled to establish the initial population. The dilutions, number of positive large/ small wells (--/--) and MPN are shown in Table 4-6 below with total coliform on the left and *E.coli* on the right.

Table 4-6: Dilutions Used for Pilot Experiment 2 and the MPN for Both Total Coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total coliform MPN	*E.coli*	MPN	*E.coli* MPN
0	**250**	**49/20**	**344.8**	= 86200	**32/2**	**52.1**	= 13025
1	200	45/14	152.9	= 30580	26/8	47.4	= 9480
2	100	32/5	57.3	= 5730	13/3	18.3	= 1830
3	50	2/0	2	= 100	0/0	<1	< 50
4	25	0/0	<1	< 25	0/0	<1	< 25
5	10	0/0	<1	< 10	0/0	<1	< 10
6	6.25	0/0	<1	< 6.25	0/0	<1	< 6.25
7	5	0/0	<1	< 5	0/0	<1	< 5
8	1.25	2/1	3	= 3.75	0/0	<1	< 1.25
9	1	4/0	4.1	= 4.1	0/0	<1	< 1
Control	250	37/5	73.3	= 18325	18/3	25.6	= 6400

Pilot experiment 2 recorded nearly twice as many positive results when compared to pilot experiment 1. However further revisions of the dilutions were required as there were still not positive readings for all the samples taken. The control result showed that both total coliform populations reduced over the course of the experiment without UV irradiance.

4.1.3 Pilot experiment 3

Pilot experiment 3 was carried out with further improvements to the dilutions used in the first two pilot experiments. The SODIS water was mixed according to Table 4-5 as per the previous pilot experiment. The irradiation of the bottles and sampling procedure from pilot experiment 2 was repeated, with the results shown in Table 4-7.

Table 4-7: Dilutions and Results of Pilot Experiment 3

Bottle	Dilution	Total coliform	MPN	Total coliform MPN	*E.coli*	MPN	*E.coli* MPN
0	**250**	**49/17**	**290.9**	= 72725	**20/1**	**26.2**	= 6550
1	200	45/4	112.6	= 22520	19/1	24.6	= 4920
2	50	43/12	124.6	= 6230	16/2	21.3	= 1065
3	1	49/48	2420	> 2420	49/22	387.3	= 387.3
4	1	49/47	2419.6	= 2420	41/11	107.1	= 107.1
5	1	49/41	1203.3	= 1203	37/4	71.2	= 71.2
6	1	49/44	1553.1	= 1553	21/2	29.2	= 29.2
7	1	49/40	1119.9	= 1120	15/1	18.7	= 18.7
8	1	49/42	1299.7	= 1300	12/3	16.9	= 16.9
9	1	48/31	456.9	= 457	15/2	19.9	= 19.9
Control	250	48/18	248.9	= 62225	19/3	25.6	= 6400

The obvious difference between experiment 3 and the previous experiments is the reduction in pathogens. The first two experiments achieved > 99.99% (4 Log), whereas experiment 3 achieved < 99.9% (3 Log). This is a substantial reduction in pathogen inactivation likely caused by the water source. As previously stated in Section 2.7.1, the source of the water can have a significant influence on the result of the SODIS method. In this instance, the variation occurred within the water source. With variations this large, determining the influence of different water constituents on the SODIS method with any certainty would be difficult.

Final improvements from pilot experiments

With the natural variation occurring in the water from the Okeover stream, a stable water source was required in order to obtain meaningful results. As the Okeover water was already being

doctored with seawater to increase the conductivity and mimic the effects of saltwater intrusion, it was decided to replace the Okeover water with de-ionised water.

4.1.4 Dominant form of inactivation

The temperature of the experimental bottles was recorded as they were sampled to determine the dominant form of the pathogen inactivation. The results for pilot experiment 2 and 3 are listed in Table 4-8.

Table 4-8: Temperature Readings (°C) for Experimental Bottles and Dark Storage Bottles at Time (h) of Bottle Removal for Sampling.

	Temperature of bottles under UV light (°C)									
Time(h)	0	1	2	3	4	5	6	7	8	9
Exp 1	19.6	23.2	26.3	28.3	30.2	31.1	31.4	33.1	32.9	32.7
Exp 2	21.6	24.5	26.8	28.4	30.5	30.8	31.9	32.7	32.4	33
	Temperature of dark storage bottles (°C)									
Time(h)	0	1	2	3	4	5	6	7	8	9
Exp 1	19.6	21	22	23	24	25.4	26	26.5	26.6	27
Exp 2	21.6	22.5	23.5	24.1	24.9	25.3	26.2	25.8	26.3	26

The maximum temperature reached by the irradiated bottles was 33°C. This was less than the recommended maximum temperature of 42 °C to ensure only optical inactivation occurs. Therefore, the pathogenic inactivation was predominantly by irradiation of UV light and not by temperature.

4.2 Total coliform experiments

From the experiments that were run, two sets of results were obtained. The results for total coliform are in this section, while the results for *E.coli* are in Section 4.3. The results for total coliform were plotted Log (C/C_0) vs Fluence. Linear approximations of the raw data were fitted based on the least squares method. The fit of the linear approximation was reviewed in combination with the residuals plot, the residuals being the difference between actual data points and the predicted data points for a given fluence. The final inactivation rate was desired, therefore, lag points at the start of the experiments were omitted to ensure the linear approximations were a good fit. All raw data can be viewed in Appendices B through I.

4.2.1 Specific conductance of 400 µS/cm

Five separate SODIS experiments were carried out with a SC of 400µS/cm, pH of 6.5 and a total hardness of 40mg/L $CaCO_3$. The results for these SODIS experiments are shown in Figure 4-1.

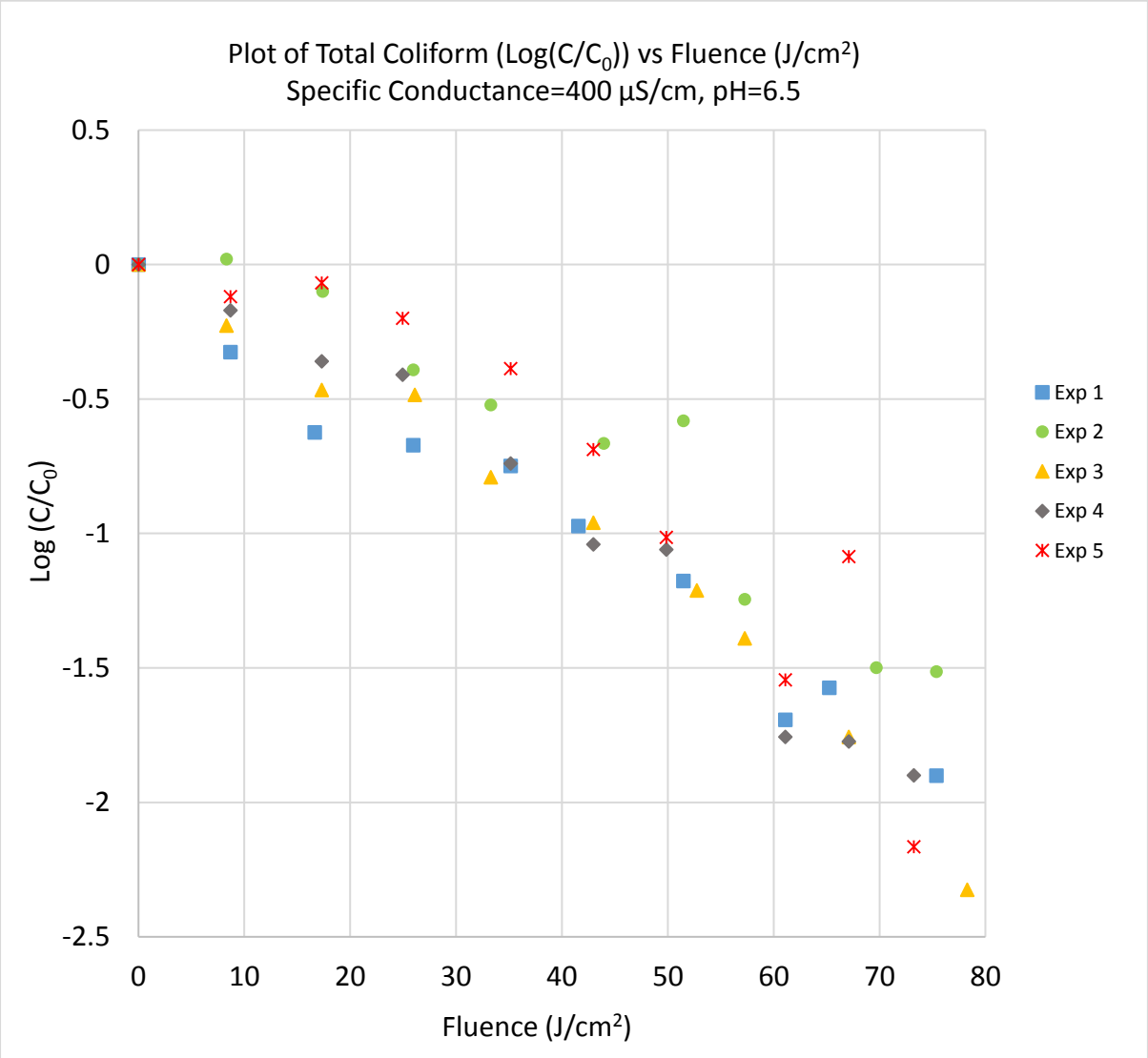

Figure 4-1: Plot of Total Coliform Reduction with Respect to Fluence for a Specific Conductance of 400µS/cm and pH = 6.5

Figure 4-1 shows a clear trend of decreasing Log(C/C_0) values on the y-axis for increasing fluence on the x-axis. This indicates that the total coliform population is decreasing with continued irradiance. Experiment 3 recorded the highest overall total coliform reduction with - 2.3log or 99.5% for a fluence of 78 J/cm^2. The least reduction in total coliform occurred from experiment 2 with ~1.5 log or 96.9% after a fluence of 75 J/cm^2. Experiment 2 and 5 show a clear lag at the beginning of the experiment before becoming approximately linear. These lag data points were removed before modelling, as the rate of inactivation rather than a one off

performance was being determined. To find the rate of inactivation, a linear approximation was fitted to the results data as shown in Figure 4-2.

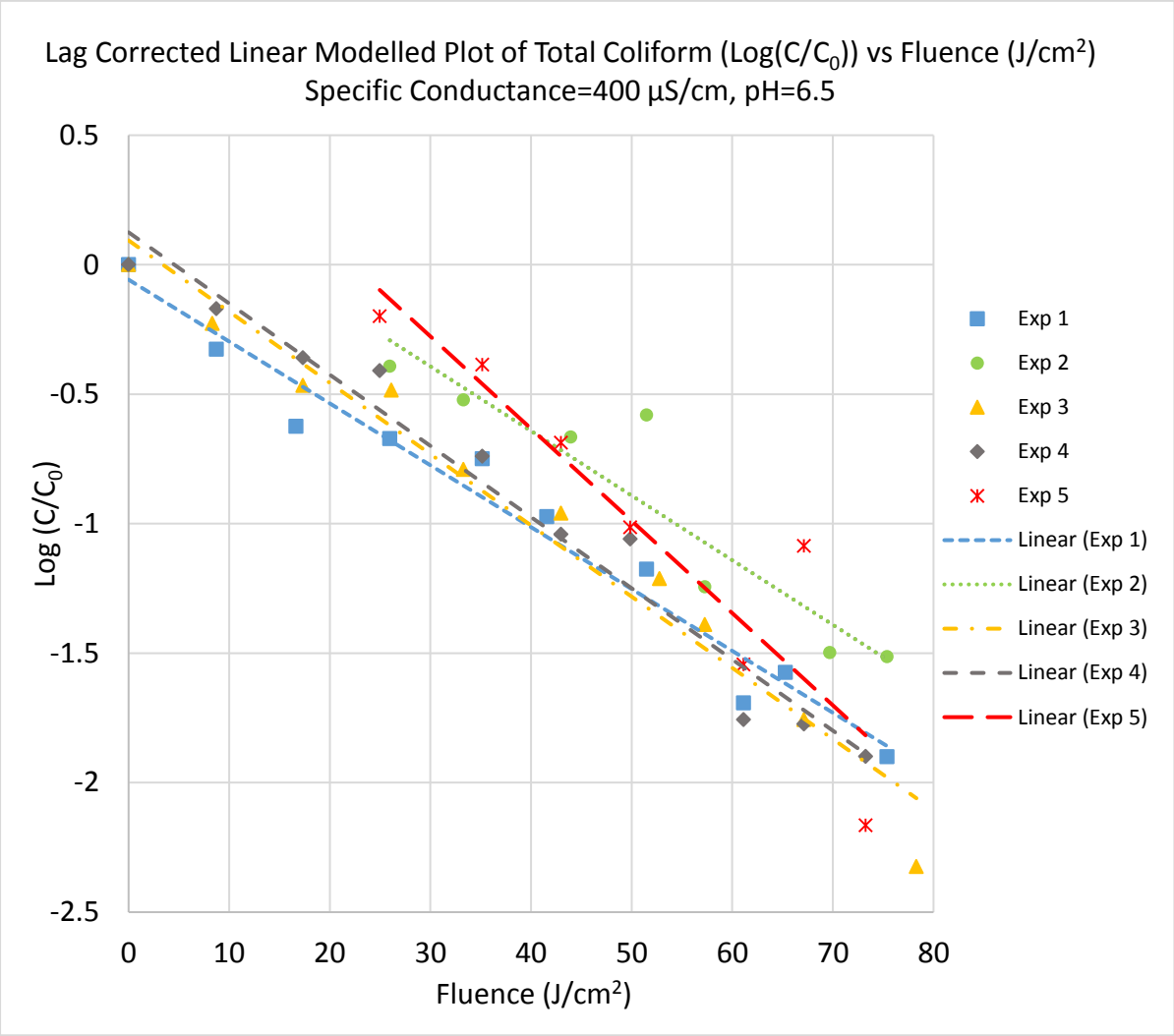

Figure 4-2: Lag Corrected Linear Approximation of Plot of Total Coliform Reduction with Respect to Fluence for a Specific Conductance of 400µS/cm and pH = 6.5

The approximately parallel-modelled lines in Figure 4-2 illustrated the slopes of the fitted lines were all very similar. Experiment 5 had a noticeably steeper slope, which indicated a higher rate of inactivation. The inactivation coefficients for all five experiments were found using (7) and are displayed in Table 4-9, along with the mean, standard error (SE) and R^2 values.

Table 4-9: Results for Slope Coefficient from Conductivity Experiment at 400 µS/cm and pH = 6.5

	Mean	S.E.	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5
k_1 (cm^2/kJ)	25	2	24	22	28	28	36
R^2	-	-	0.97	0.87	0.97	0.97	0.84
Skew = 1.58 < 2.2, so data is negatively skewed but not significantly							

The skew value of 1.58 in Table 4-9 indicated that the data was positively skewed towards higher values. This was explained by experiment 5 with a slope value approximately 1.5x higher than the mean. As the skew value was less than the significance value of 2.2 the data was not considered significantly skewed. Therefore, the data was assumed to be normally distributed and further statistical analysis carried out. The high R^2 values indicated a good fit by the linear approximations to the actual data.

4.2.2 Specific conductance of 700 µS/cm

Three independent SODIS experiments were carried out with a conductivity of 700µS/cm, pH of 6.7 and a total hardness of 62mg/L $CaCO_3$. The results for these SODIS experiments are shown in Figure 4-3

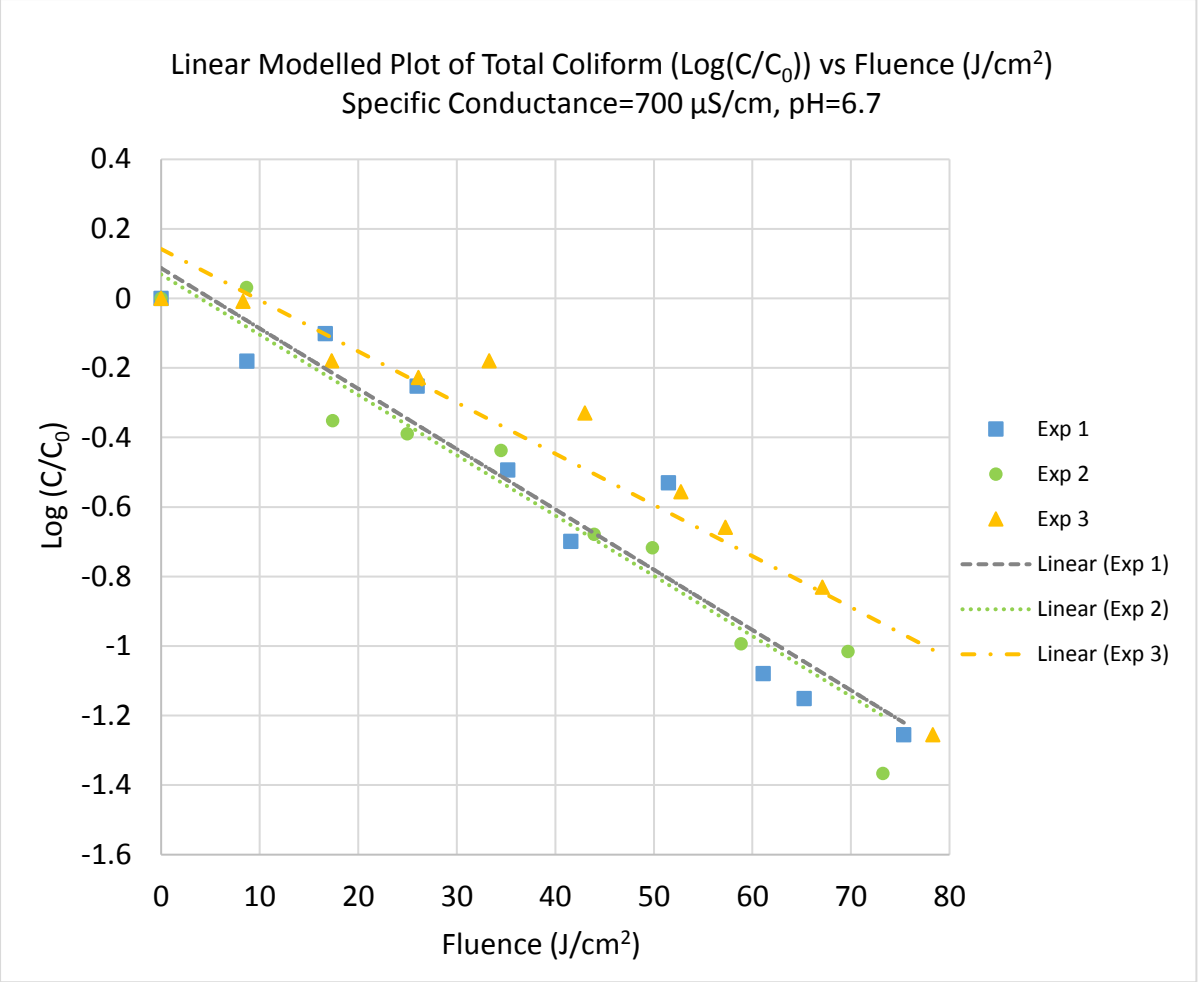

Figure 4-3: Linear Approximation of Plot of Total Coliform Reduction with Respect to Fluence for a Specific Conductance of 700µS/cm and pH = 6.7

There was a clear trend in the data of reducing total coliform population with increasing fluence shown in Figure 4-3. Results from experiment 1 and 2 had different data scatters but were nearly identical when modelled with a linear approximation. Experiment 3 had a slower inactivation, which was evident in the modelled line, had a flatter slope. The slope values are displayed in Table 4-10 with the mean, SE and R^2 values.

Table 4-10: Results for Slope Coefficient from Conductivity Experiment at 700 µS/cm and pH = 6.7

	Mean	S.E.	Exp 1	Exp 2	Exp 3
k_1 (cm^2/kJ)	16	1	17	17	14
R^2	-	-	0.92	0.95	0.90
Skew	= -1.73 < 2.83 so data is negatively skewed but not significantly				

The high negative skew of -1.73 from Table 4-10 occurred due to experiment 3 having a lower slope value than the other two experiments, which were the same. This skew was not significant so a normal distribution was assumed for statistical analysis. The high R^2 values indicated a good fit by the linear approximations to the actual data.

4.2.3 Specific conductance of 900 µS/cm

To investigate the effect of high conductivity on the SODIS method, four experiments were independently conducted. The SC was set at 900 µS/cm with the pH and total hardness being recorded as 6.8 and 78mg/L $CaCO_3$ respectively. The results from these experiments are displayed in Figure 4-4.

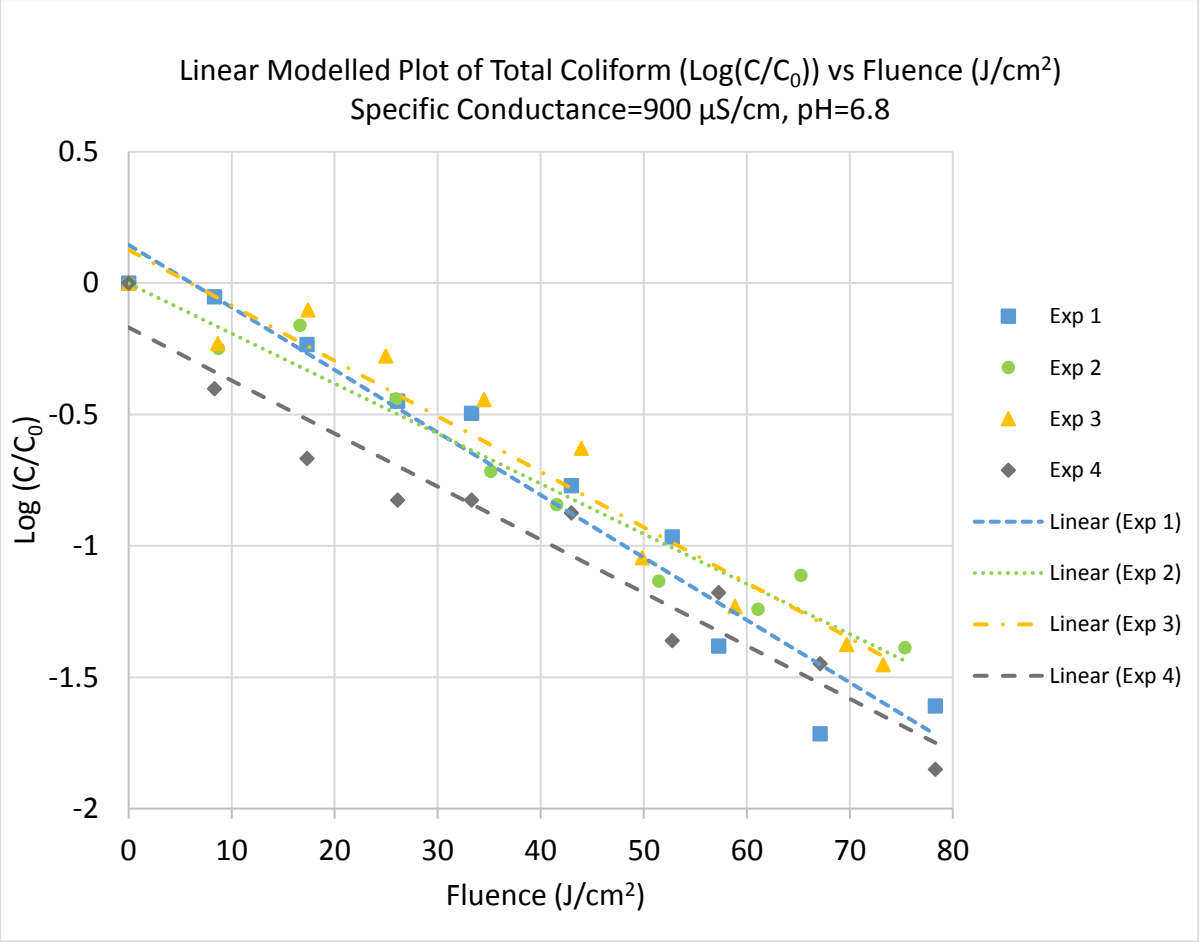

Figure 4-4: Linear Approximation of Plot of Total Coliform Reduction with Respect to Fluence for a Specific Conductance of 900µS/cm, pH = 6.8 and Total Hardness = 78mg/L $CaCO_3$

The results data in Figure 4-4 was well distributed with no obvious lags or tails. A clear trend of decreasing total coliform population with increasing fluence was observed. The linear approximations were a good fit and similar with only experiment 1 having a different slope. Table 4-11 lists the calculated slope values, R^2 values, mean and SE for the modelled lines.

Table 4-11: Results for Slope Coefficient from Conductivity Experiment at 900 µS/cm and pH = 6.8

	Mean	S.E.	Exp 1	Exp 2	Exp 3	Exp 4
k_1 (cm^2/kJ)	21	1	24	19	21	20
R^2	-	-	0.95	0.96	0.94	0.94
Skew	= 1.05 < 2.45 so data is positively skewed but not significantly					

The slope values in Table 4-11 were closely grouped as was seen in Figure 4-4 with the exception of experiment 1 being noticeably higher. This higher slope value positively skewed the data with the skew = 1.05. The skew was still below the significance threshold, so the data was treated as normally distributed. The high R^2 values indicated a good fit by the linear approximations to the actual data.

4.2.4 pH = 8.3 and specific conductance of 400 µS/cm

The ground water in Kiribati has a typical pH = 8.3. Therefore, three experiments were carried out with a SC of 400 µS/cm at this higher pH of 8.3 to record any effect on pathogen inactivation. The total hardness of 38 mg/L $CaCO_3$ was unchanged from the previous SC= 400 µS/cm experiment. The findings from the experiments are shown in Figure 4-5

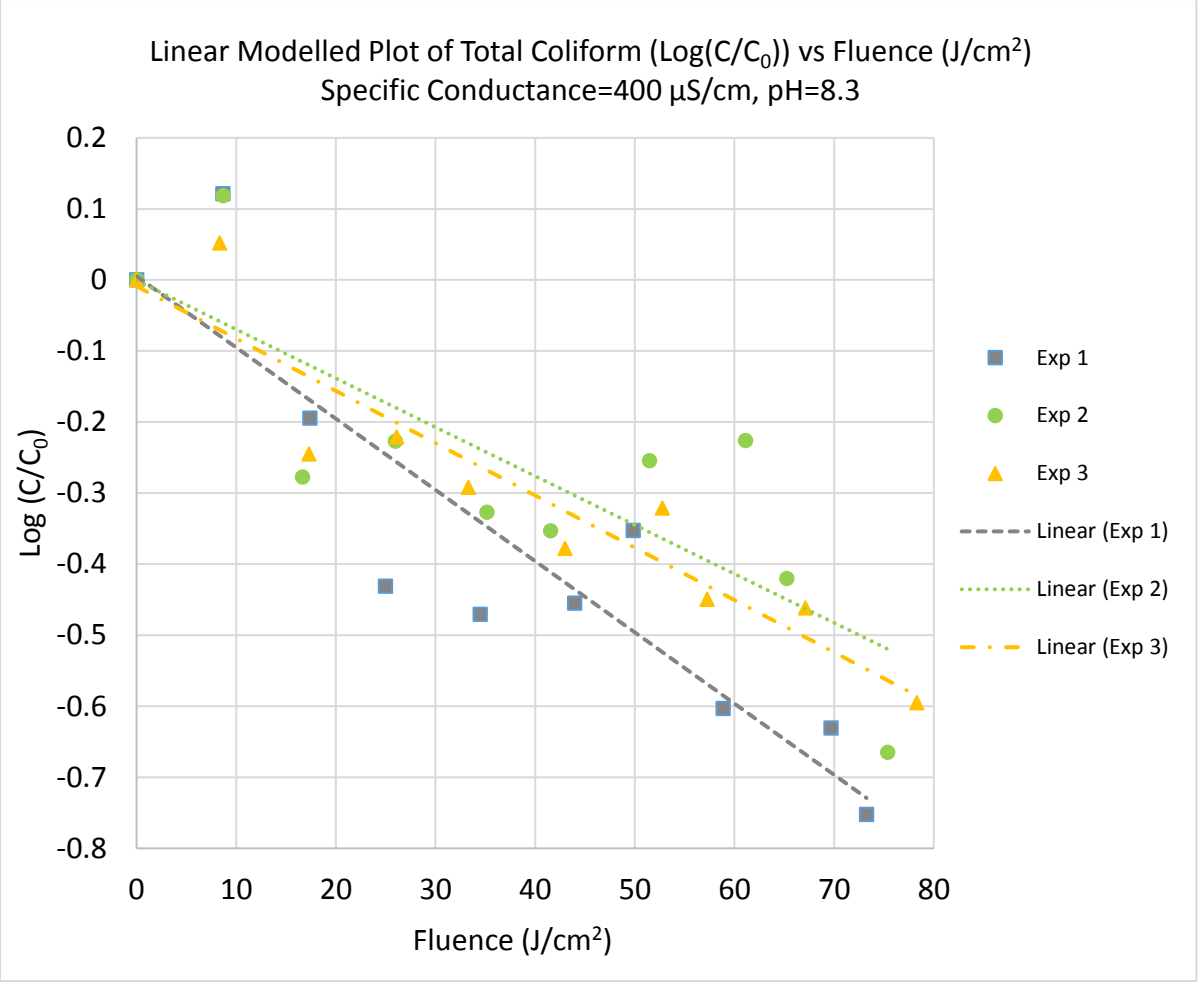

Figure 4-5: Linear Approximation of Plot of Total Coliform Reduction with Respect to Fluence for a Specific Conductance of 400µS/cm and pH = 8.3

The first observation from Figure 4-5 was the poor performance with a maximum reduction of only log 0.75 or ~82%. All three experiments initially experienced growth in total coliform populations as indicated by the positive values. Experiment 1 followed the initial growth period by a rapid inactivation, a plateauing, an increase in numbers, before finishing with a linear reduction. Experiment 2 showed a similar pattern to experiment 1 with a rapid reduction, plateauing in the middle of the experiment and an increase in numbers before finishing on a rapid decrease. This growth and decay pattern of experiments 1 and 2 resulted in a poor visual fit of the linear approximation. The slope, mean, SE and R^2 values of the three experiments are shown in Table 4-12.

Table 4-12: Results for Slope Coefficient from Conductivity Experiment at 400 µS/cm and pH = 8.3

	Mean	S.E.	Exp 1	Exp 2	Exp 3
k_1 (cm^2/kJ)	8	1	10	7	7
R^2	-	-	0.83	0.66	0.89
Skew	= 1.56 < 2.83 so data is positively skewed but not significantly				

Table 4-12 confirmed the poor fit of the linear approximations to the data. Experiment 2 had R^2 values of only 0.66. Experiment 1 with its higher value for slope skewed the data positively with a skew = 1.56. This was less than the level for significance so the data was treated as normally distributed.

4.2.5 pH = 8.3 and specific conductance of 700 µS/cm

The results from the three experiments with SC = 700 µS/cm, the higher pH = 8.3 and a total hardness of 62mg/L $CaCO_3$ are displayed on the plot in Figure 4-6.

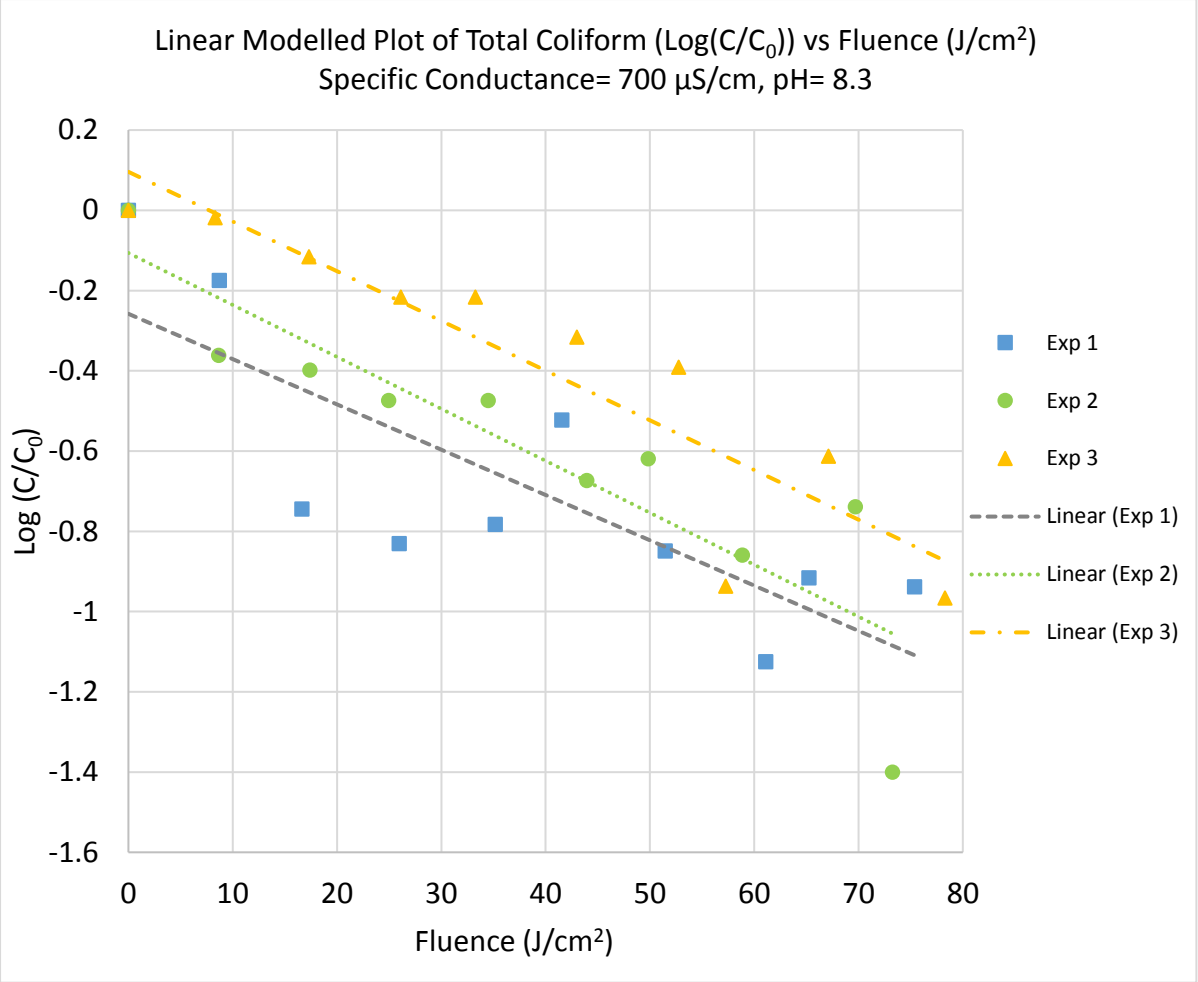

Figure 4-6: Linear Approximation of Plot of Total Coliform Reduction with Respect to Fluence for a Specific Conductance of 700µS/cm and pH = 8.3

The results data in Figure 4-6 looked well distributed for experiments 2 and 3 with a final inactivation of -1.4 log or 96%. Experiment 1 had considerable scatter and showed growth in total coliform around a fluence of 40 J/cm^2. The slopes of the fitted linear lines all appeared parallel, which indicated that they all had similar inactivation coefficients. These coefficients are displayed in Table 4-13 with the R^2 values, mean and SE.

Table 4-13: Results for Slope Coefficient from Conductivity Experiment at 700 µS/cm and pH = 8.3

	Mean	S.E.	Exp 1	Exp 2	Exp 3
k_1 (cm^2/kJ)	12	<1	11	13	12
R^2	-	-	0.65	0.79	0.83
Skew	= -1.03 < 2.83 so data is negatively skewed but not significantly				

The skew of -1.03 from Table 4-13 indicated that the data was not significantly skewed. From a visual check of the slope values, it would be expected the skew should be zero, this was due to the level of accuracy that could be reported and the rounding associated with it. Due to the close values for the inactivation coefficients the SE error is small (<1 cm^2/kJ).

4.2.6 pH = 8.3 and specific conductance of 900 µS/cm

Three experiments with SC of 900 µS/cm, pH=8.3 and a total hardness of 78 mg/L $CaCO_3$ were carried out to investigate the effect of high pH and high conductivity on the SODIS method. The results are shown in Figure 4-7.

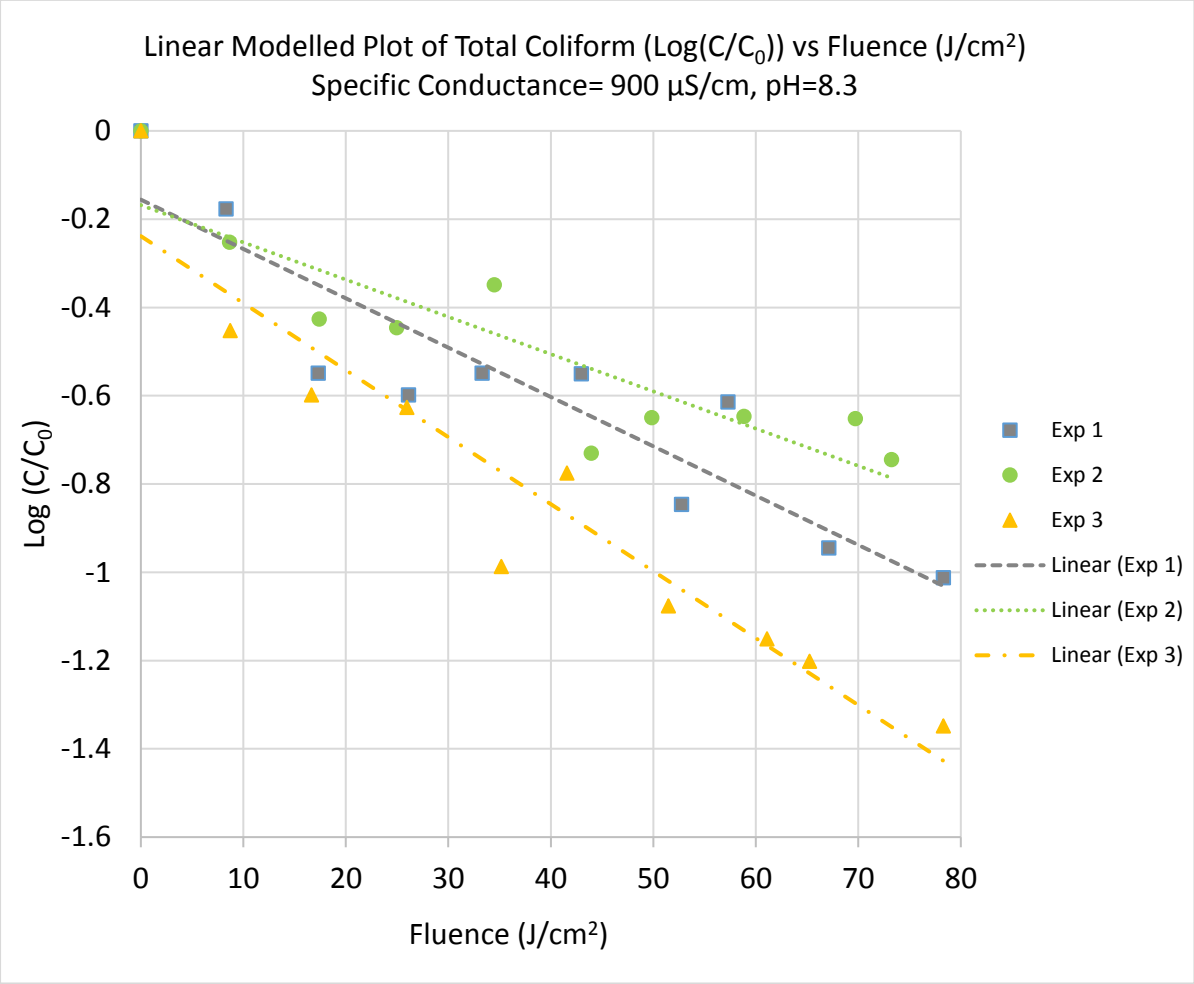

Figure 4-7: Linear Approximation of Plot of Total Coliform Reduction with Respect to Fluence for a Specific Conductance of 900µS/cm and pH = 8.3

A large variation in the overall inactivation of the experiments was seen in Figure 4-7. The maximum and minimum inactivation were 1.4log and 0.8log respectively. Fairly even scatter patterns were seen with different slopes being realised on the fitted lines. The mean of the slope values, SE, R^2 and actual values are shown in Table 4-14.

Table 4-14: Results for Slope Coefficient from Conductivity Experiment at 900 µS/cm and pH = 8.3

	Mean	S.E.	Exp 1	Exp 2	Exp 3
k_1 (cm^2/kJ)	12	2	11	8	15
R^2	-	-	0.84	0.79	0.91
Skew	= 0.52 < 2.83 so data is positively skewed but not significantly				

Even with the variety of slopes in Table 4-14, the skew value of 0.52 indicated that they were close to being symmetrical about the mean. However, the range in slope values did result in a large value for the SE of 17% of the mean. The wide scatter also affected the fit of the linear approximation to the data with two of the three values being < 0.85.

4.2.7 Total coliform – water hardness

Kiribati being a coral atoll and having saltwater intrusion into its freshwater lens has high water hardness. The following three experiments were carried out to investigate the effect of high total hardness on the SODIS method. The results from these experiments are shown in Figure 4-8.

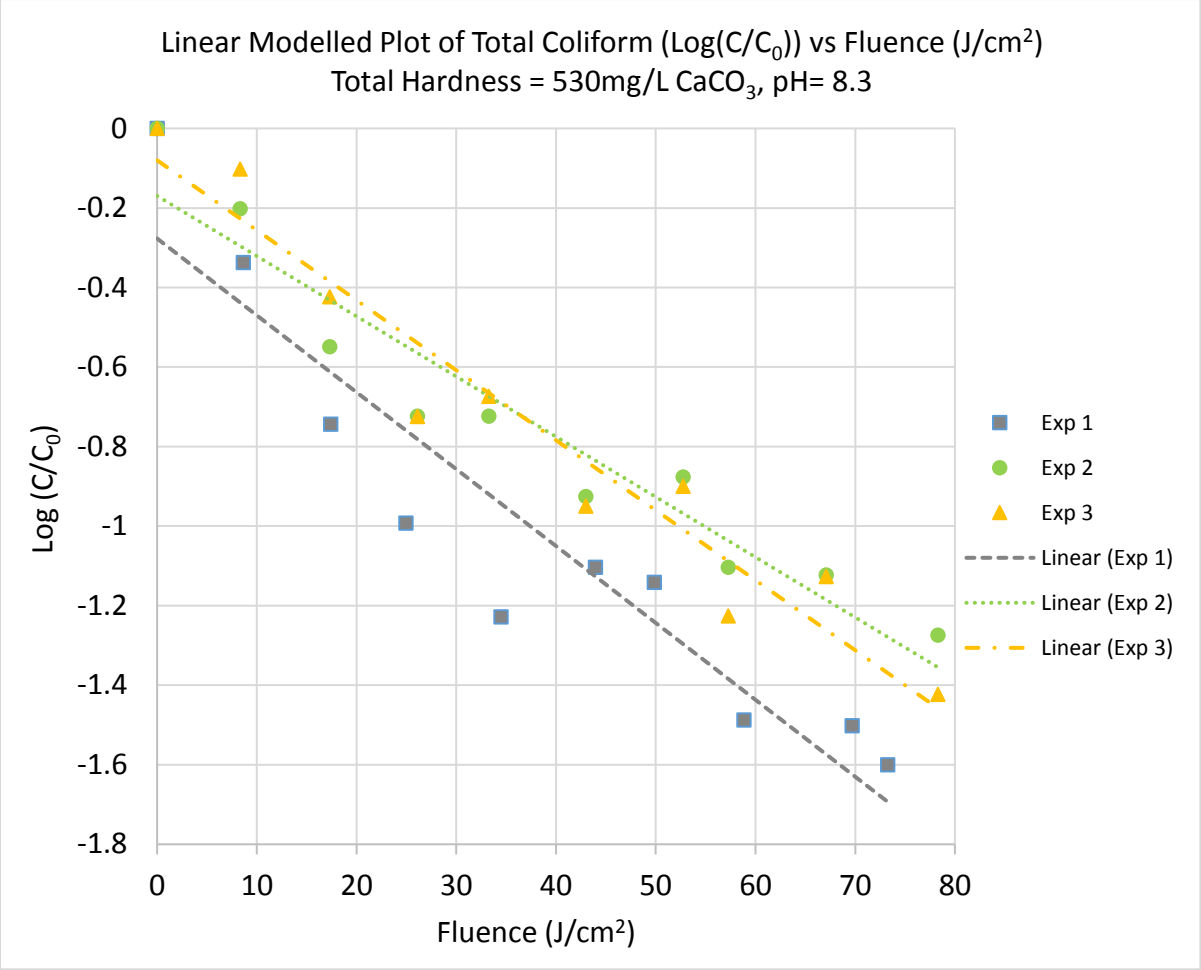

Figure 4-8: Linear Approximation of Plot of Total Coliform Reduction with Respect to Fluence for a Total Hardness = 530mg/L $CaCO_3$, Specific Conductance = 1830µS/cm and pH = 8.3

The scatter of the data points for all experiments shown in Figure 4-8 were well grouped and had a good overall inactivation. The final inactivation were between 1.6log and 1.3log. The linear modelled lines fitted the data and had similar slopes across the experiments. The values for the slopes, R^2, mean and SE are displayed in Table 4-15.

Table 4-15: Results for Slope Coefficient with Total Hardness >550mg/L $CaCO_3$ and pH = 8.3

	Mean	S.E.	Exp 1	Exp 2	Exp 3
k_1 (cm^2/kJ)	17	1	19	15	18
R^2	-	-	0.88	0.92	0.94
Skew	= -0.56 < 2.83 so data is negatively skewed but not significantly				

The slope values from Table 4-15 were similar with a good distribution, this resulted in minimal negative skew = -0.56 and a SE ~5% of the mean. Good values for R^2 were recorded indicating the linear approximations fitted the data well.

4.2.8 Total coliform - bottle size

The influence of water depth on the SODIS method was explored by reducing the size of the bottles used in the experiment from 1.5L bottles to 0.355L bottles. The characteristics of these bottles was measured from a sample of three bottles and the results are shown in Table 4-16.

Table 4-16: Comparison of Characteristics for Different Bottle Sizes used for SODIS Experiments

	Large Bottle	Small Bottle
Diameter (mm)	93	60
Mass (g)	40.83	22.61
Volume (L)	1.50	0.355
Mass / Volume (g/L)	27.2	63.7
UV transmission (mW/cm^2) (baseline reduction)	0.305 (44%)	0.342 (37%)

Using the 60mm diameter bottles, three experiments were conducted using the same solution as per the hard water experiments. The inactivation results are shown in Figure 4-9 together with the linear approximations.

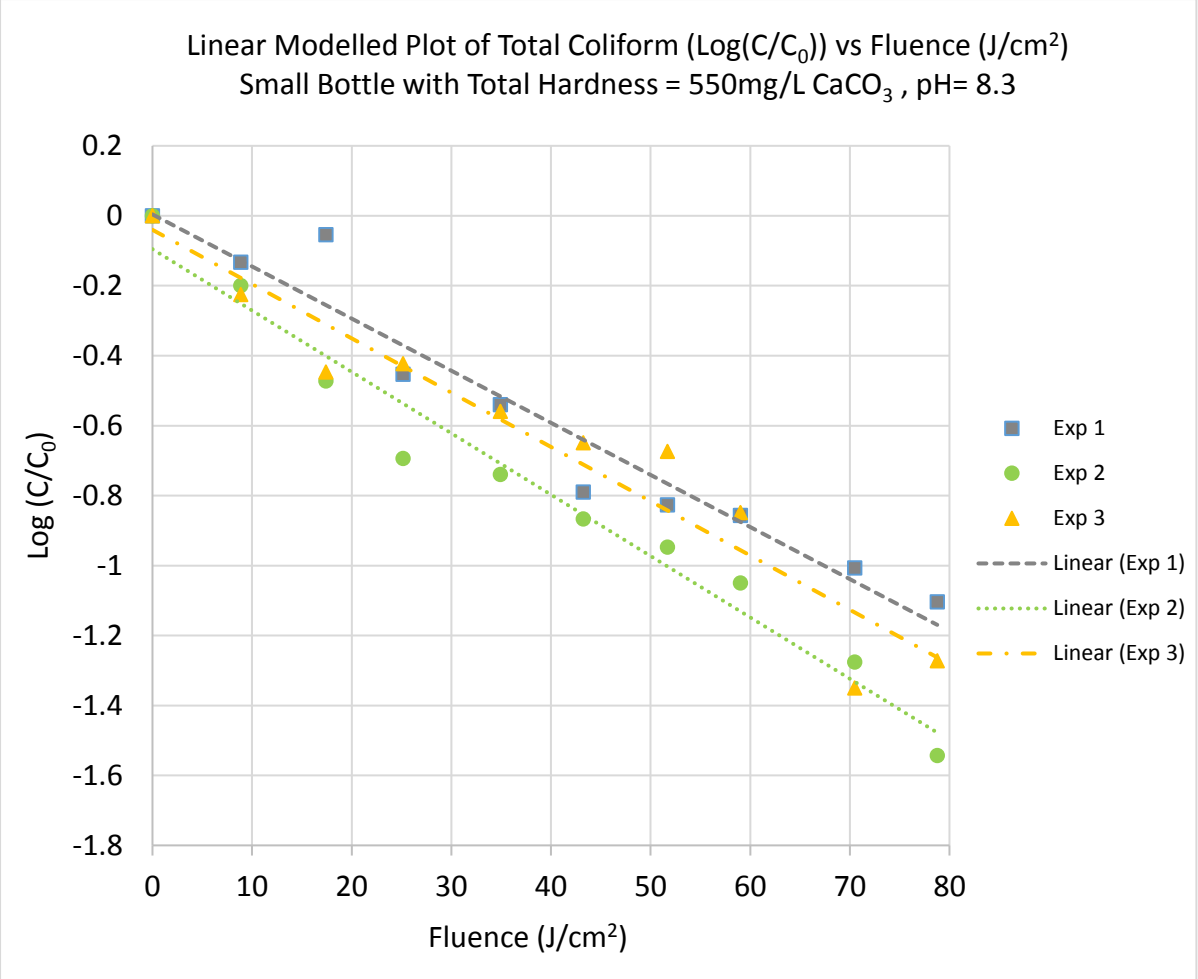

Figure 4-9: Linear Approximation of Plot of Total Coliform Reduction with Respect to Fluence for a Small Bottle, Total Hardness =550mg/L $CaCO_3$, Specific Conductance = 1830µS/cm and pH = 8.3

The results in Figure 4-9 had the tightest grouping of data when compared to the previous experiments. This resulted in a very good fit of the linear approximation. Consistent inactivation outcomes of between 1.1 log and 1.6 log were realised.

Table 4-17: Slope Coefficients for Small Bottles with Total Hardness >550mg/L $CaCO_3$ and pH = 8.3

	Mean	S.E.	Exp 1	Exp 2	Exp 3
k_1 (cm^2/kJ)	16	<1	15	18	16
R^2	-	-	0.94	0.97	0.93
Skew	= 1.36 < 2.83 so data is positively skewed but not significantly				

The three slope results in Table 4-17 had a small spread, which resulted in the SE ~ 5% of the mean. The data was represented well by the linear approximations with very high R^2 values. Although the slope values at 1.36 were positively skewed, this was not statistically significant. Therefore, further statistics requiring normal distribution were possible.

4.2.9 Total coliform overall discussion

A combination of all the inactivation results from the total coliform experiments in Section 4.2 were plotted on the box and whisker diagram in Figure 4-10. The box size is set by the upper and lower quartiles of the results with the horizontal line being the median value. The whiskers represent the maximum and minimum values from the data. Statistical significance of the difference in inactivation constants was carried out using an unpaired T test assuming equal variance with the results shown in Table 4-18.

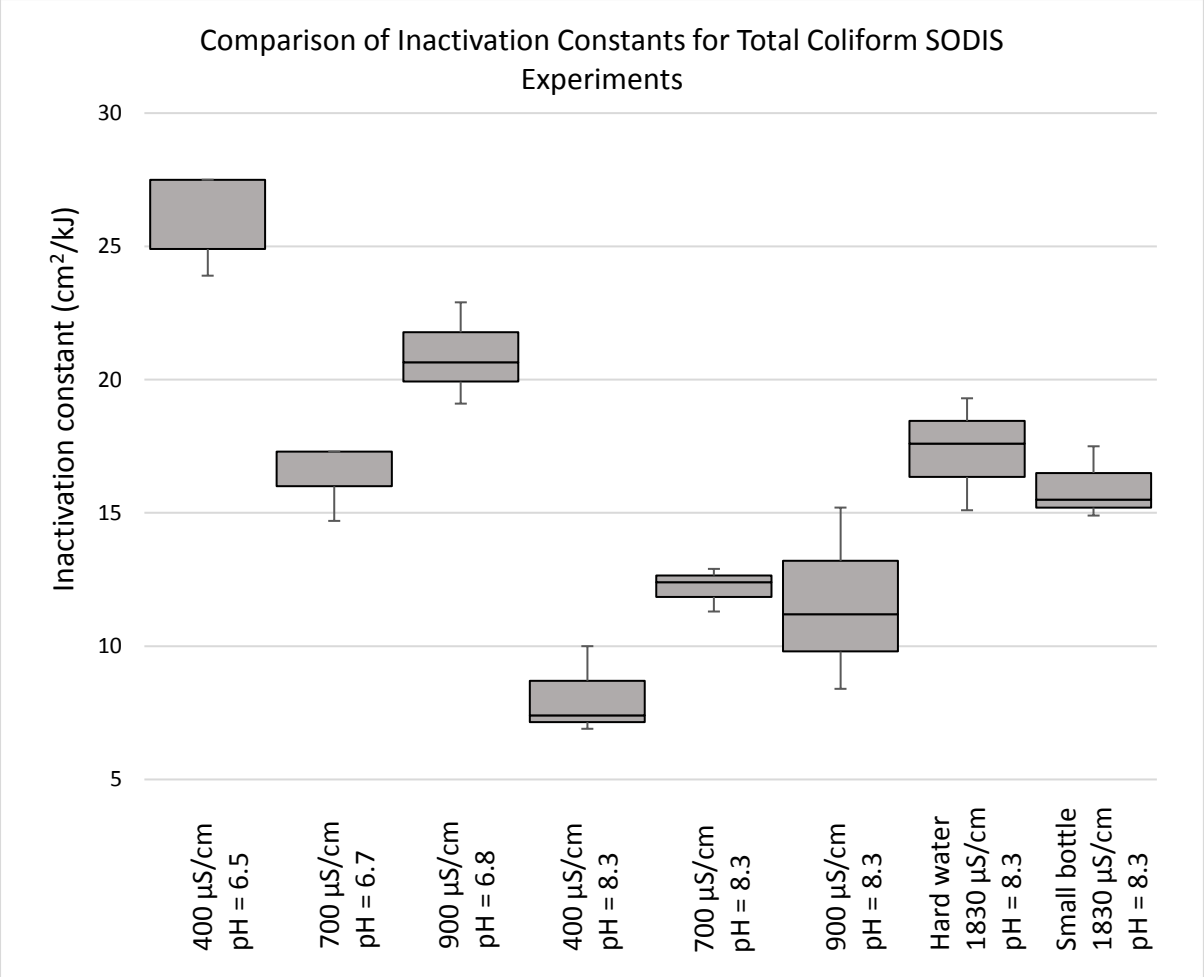

Figure 4-10: Box and Whisker Plot of Total Coliform Inactivation Constants (cm^2/kJ)

Table 4-18: Statistical Significance of the Mean Slope for Various Total Coliform SODIS Experiments

(p = 0.05)	400µS/cm pH= 6.5	700µS/cm pH= 6.7	900µS/cm pH= 6.8	400µS/cm pH= 8.3	700µS/cm pH= 8.3	900µS/cm pH= 8.3	Hard 1830µS/cm pH= 8.3
700µS/cm pH= 6.7	0.007 = Significant						
900µS/cm pH= 6.8	0.029 = Significant	0.021 = Significant					
400µS/cm pH= 8.3	<0.001 = Significant	0.015 = Significant	<0.001 = Significant				
700µS/cm pH= 8.3	0.001 = Significant	0.058 = **NOT** Significant	0.001 = Significant	0.103 = **NOT** Significant			
900µS/cm pH= 8.3	0.002 = Significant	0.225 = **NOT** Significant	0.006 = Significant	0.245 = **NOT** Significant	0.804 = **NOT** Significant		
Hard 1830µS/cm pH= 8.3	0.010 = Significant	0.625 = **NOT** Significant	0.064 = **NOT** Significant	0.004 = Significant	0.092 = **NOT** Significant	0.079 = **NOT** Significant	
Small bottle 1830µS/cm pH= 8.3	0.005 = Significant	0.681 = **NOT** Significant	0.013 = Significant	0.041 = Significant	0.013 = Significant	0.230 = **NOT** Significant	0.564 = **NOT** Significant

pH

From Figure 4-10 the fastest inactivation occurred when the specific conductance (SC) was 400µS/cm and pH = 6.5 with an inactivation constant ~25 cm^2/kJ. The slowest inactivation of ~ 8 cm^2/kJ also occurred with a SC = 400µS/cm but at the higher pH = 8.3. The low pH experiments appeared to all have higher inactivation than the same experiments with a higher pH. This is in agreeance with the findings of Fisher et al. (2008), who found that the inactivation of pathogens increased as pH decreased.

Conductance

The inactivation constant for experiments with SC = 900µS/cm appear to perform midway between SC 400 and 700µS/cm regardless of pH in Figure 4-10. This difference was confirmed with the statistical significance from Table 4-18 for pH< 7. However, for pH = 8.3 there is no statistical difference between the three conductance.

Hard water

The hard water results for statistical significance (p=0.05) from Table 4-18 with pH = 8.3 show that there is a significant difference to experiments carried out with pH = 8.3 and SC = 400µS/cm. This may be due to the big difference in conductance or it may be from the increase in total hardness. The hard-water experiments had a specific conductance ~ 1800µS/cm.

Bottle size

The SODIS solution used in the hard-water experiments and the small bottle experiments was characteristically the same. The only variable that changed between experiments was the water depth, which is inherent with bottle size. The larger bottles appear to have performed better with an inactivation rate of 17cm^2/kJ versus the smaller bottles at 16cm^2/kJ from Figure 4-10. Table 4-18 states that this difference in not statistically significant however, which is in direct contrast to Dessie et al. (2014) who achieved overall inactivation of faecal coliform with smaller bottles (55mm diameter) and larger bottles (100mm diameter) of ~2.9 Log and 0.5 Log respectively.

4.3 *E.coli* experiments

The results for *E.coli* from the experiments are outlined in this Section. The linear approximations to the raw data were fitted as per the total coliform experiments. All raw data information is included in the Appendices B- I.

4.3.1 Specific conductance of 400 µS/cm

All five experiments that were carried out with a SC = 400 µS/cm and pH = 6.5 resulted in significant lag with respect to E.coli inactivation. These data points were omitted so as not to affect the linear modelling as shown in Figure 4-11.

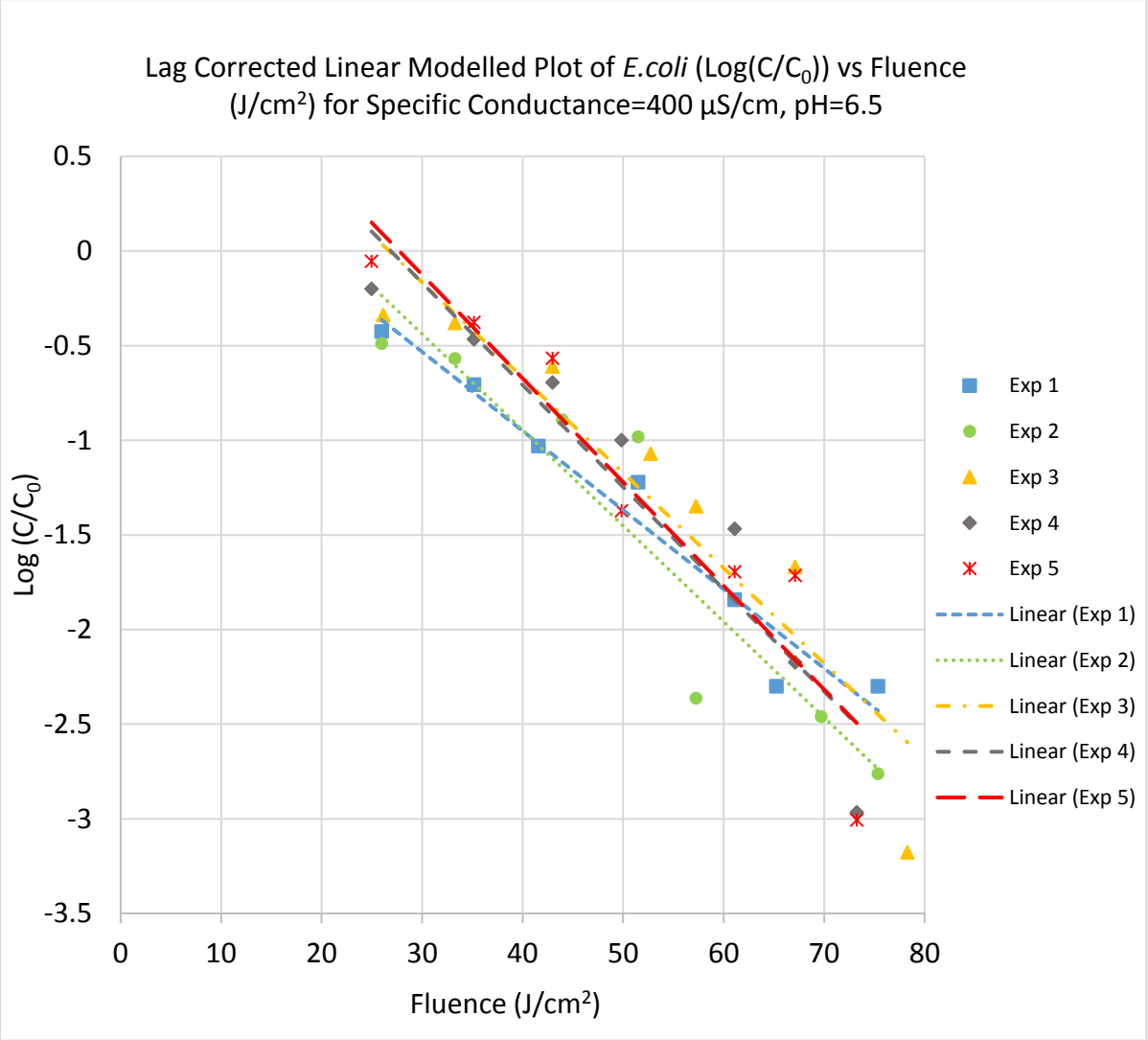

Figure 4-11: Lag Corrected Linear Approximation of Plot of E.coli Reduction with Respect to Fluence for a Specific Conductance of 400µS/cm and pH = 6.5

There was an overall trend of increasing *E.coli* inactivation (decreasing population) with increasing fluence. High inactivation of the *E.coli* was achieved with three experiments

reaching 3.0 log or higher. The linear lines appear to fit the data well with minimal outliers and have similar slopes. The values for the slopes along with the R^2, mean and SE are listed in Table 4-19.

Table 4-19: Results for Slope Coefficient for E.coli with SC of 400 µS/cm and pH = 6.5

	Mean	S.E.	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5
k_1 (cm^2/kJ)	50	2	42	51	50	54	55
R^2			0.96	0.87	0.87	0.91	0.90
Skew = -1.37 < 2.2, so data is negatively skewed but not significantly							

The skew = -1.37 from Table 4-19, indicated the data was negatively skewed but not significantly. This was likely due to experiment 1, which had a lower inactivation value than the others. The SE = 2 may seem high but was only 4% of the mean value of 50 cm^2/kJ. High R2 values indicate the linear approximations were a good fit to the data.

4.3.2 Specific conductance of 700 µS/cm

The three experiments investigating *E.coli* inactivation with SC = 700 µS/cm and pH = 6.7 all had substantial lag periods. Removal of the lag data points was necessary before linear modelling as shown in Figure 4-12.

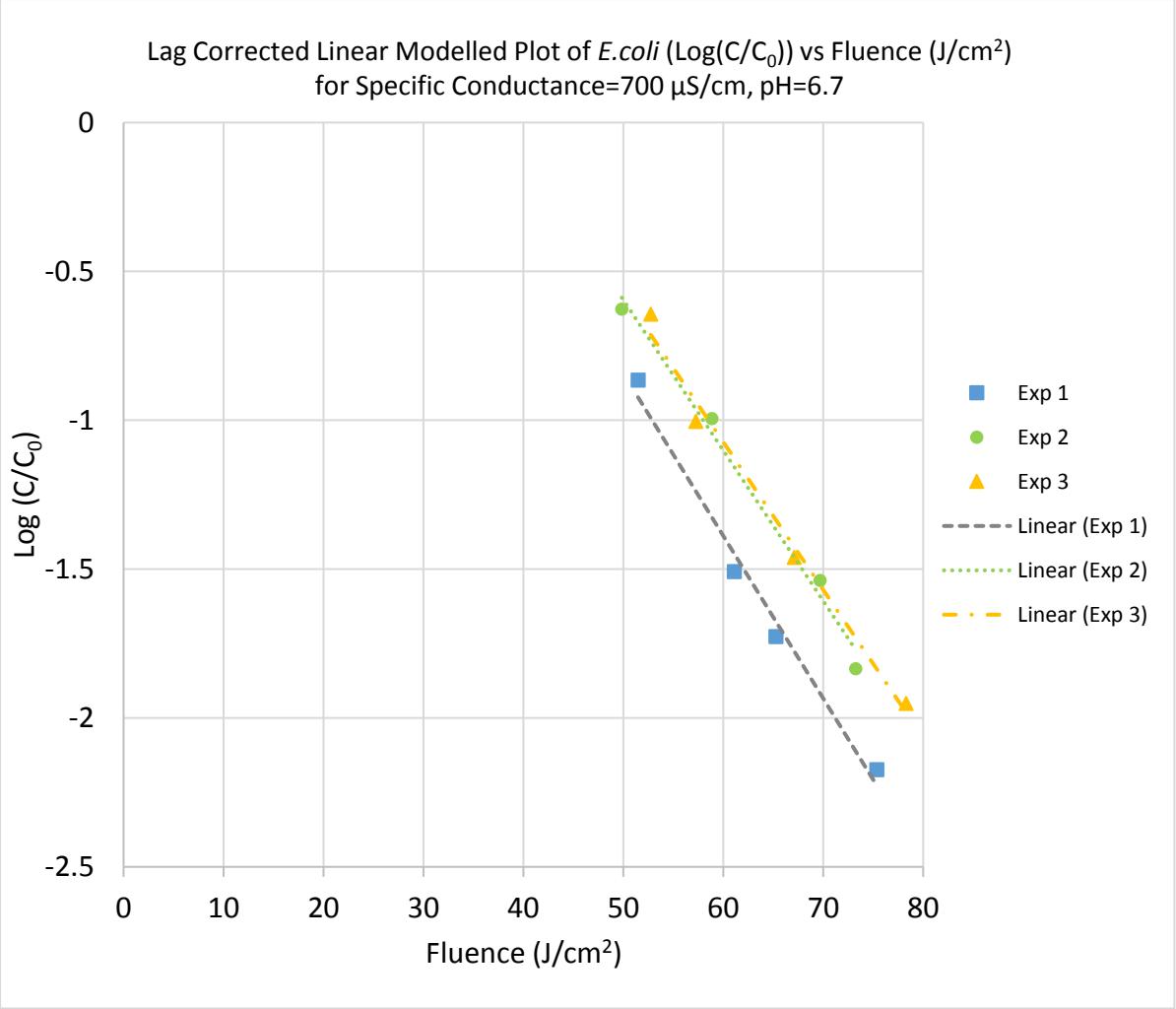

Figure 4-12: Lag Corrected Linear Approximation of Plot of E.coli Reduction with Respect to Fluence for a Specific Conductance of 700µS/cm and pH = 6.7

All the experiments show a trend of decreasing *E.coli* population with increasing fluence. The maximum *E.coli* inactivation for the three experiments was ~2.2 log. After the lag period was omitted, the remaining data points appear linear, resulting in a good fit of the linear approximations. Overall, all the experiments had similar slopes with the values being displayed in Table 4-20.

Table 4-20: Results for Slope Coefficient for E.coli with SC of 700 µS/cm and pH = 6.7

	Mean	S.E.	Exp 1	Exp 2	Exp 3
k_1 (cm^2/kJ)	52	2	55	50	50
R^2	-	-	0.99	0.99	0.99
Skew	= 1.55 < 2.83 so data is positively skewed but not significantly				

The linear approximations in Table 4-20 were an excellent fit to the data with all R^2 values = 0.99. The slope values were comparative with the SE = 2 cm^2/kJ or <5% of the mean = 52 cm^2/kJ. A skew = 1.55 indicated that the distribution was positively skewed but not significantly. This skew was caused by the result from experiment 1, which was higher than the other values.

4.3.3 Specific conductance of 900 µS/cm

Lag periods were seen in the results from the four experiments investigating *E.coli* inactivation with SC = 900 µS/cm and pH = 6.8. Removal of the lag data points was necessary before linear modelling as shown in Figure 4-13.

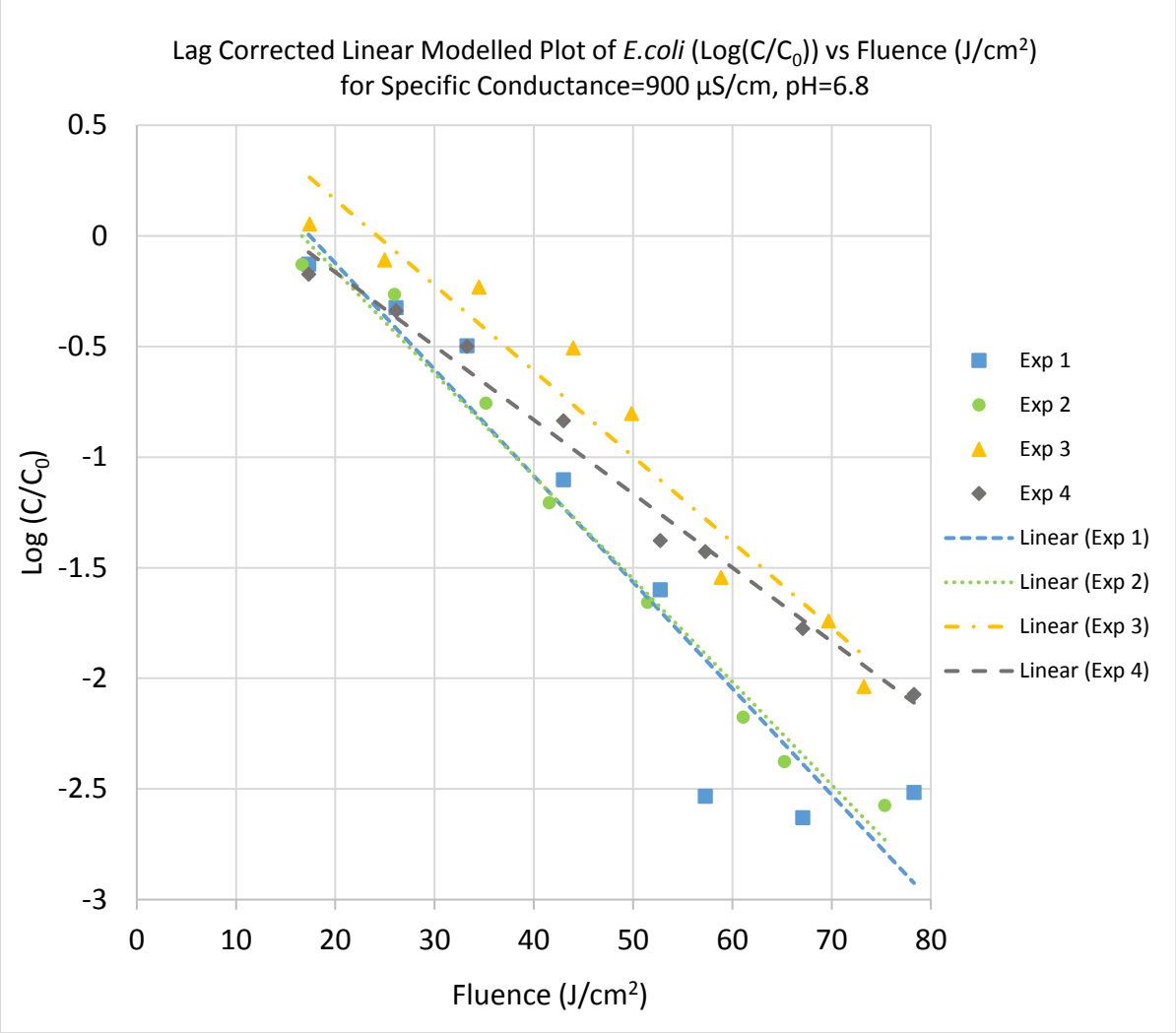

Figure 4-13: Lag Corrected Linear Approximation of Plot of E.coli Reduction with Respect to Fluence for a Specific Conductance of 900µS/cm and pH = 6.8

From Figure 4-13, there was a clear trend of reducing *E.coli* numbers with increasing fluence. Two experiments exceeded an inactivation of 2.5 log, with experiment 1 exceeding it with three readings. The remaining two experiments reached an inactivation of 2.0 log. Three of the slopes appeared similar with the slope for experiment 4 being noticeably flatter. Table 4-21 shows the slopes for the modelled lines along with the mean, SE and R^2 values.

Table 4-21: Results for Slope Coefficient for E.coli with SC of 900 µS/cm and pH = 6.8

	Mean	S.E.	Exp 1	Exp 2	Exp 3	Exp 4
k_1 (cm^2/kJ)	42	3	48	46	39	33
R^2	-	-	0.90	0.98	0.95	0.98
Skew	= -0.44 < 2.45 so data is positively skewed but not significantly					

From Table 4-21, experiment 4 had the smallest slope with a value of 33 cm^2/kJ but a good fit to the data with R^2 = 0.98. This negatively skewed the population with a skew = -0.44, although this was not statistically significantly. Due to the large diversity in slope values, the SE was ~7% of the mean.

4.3.4 pH = 8.3 and specific conductance of 400 µS/cm

The results from the three experiments investigating *E.coli* inactivation with SC = 400 µS/cm and pH = 8.3 are shown in Figure 4-14 with linear approximations fitted to the data. There were no obvious lag for these experiments so all the data points were plotted.

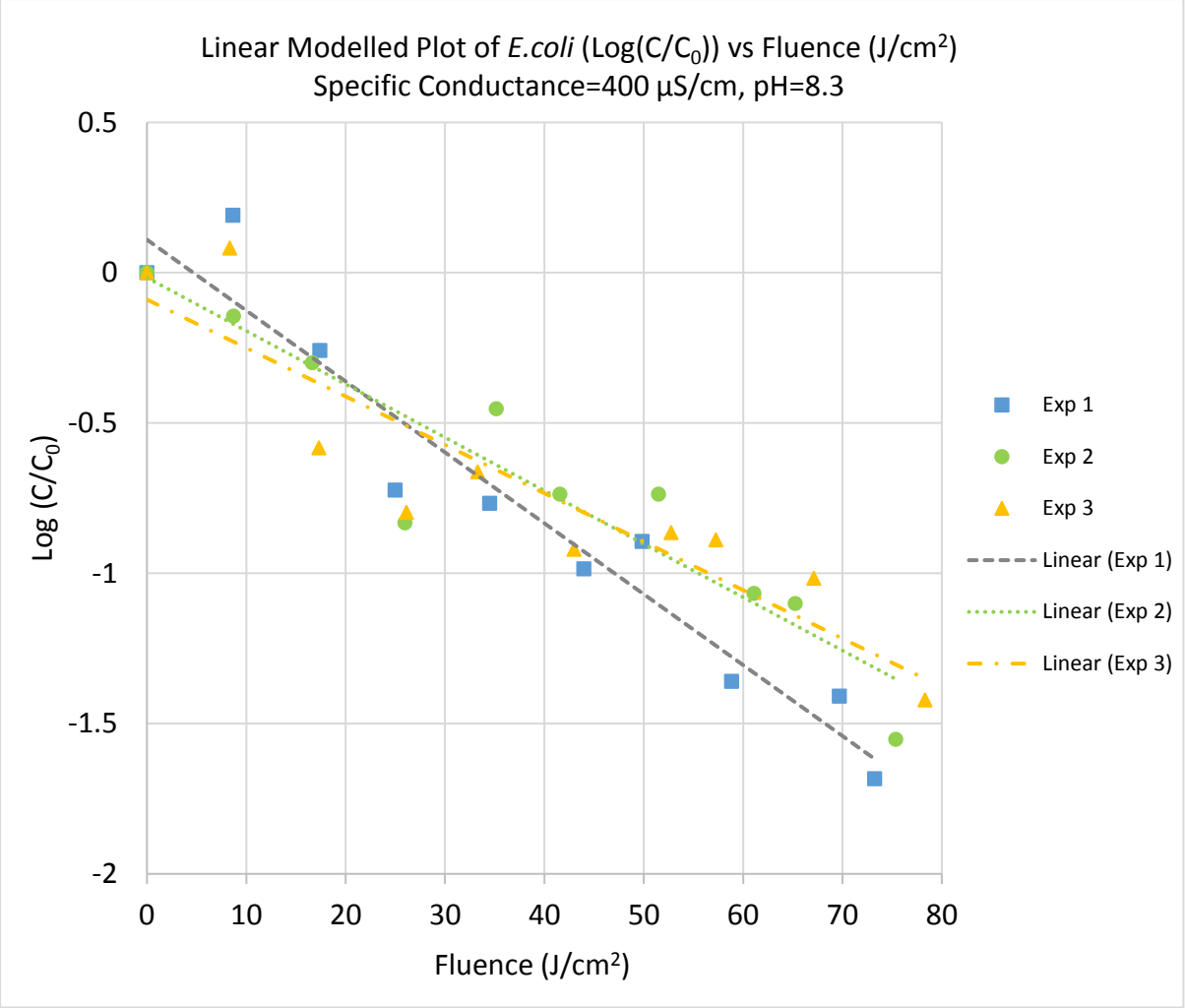

Figure 4-14: Linear Approximation of Plot of E.coli Reduction with Respect to Fluence for a Specific Conductance of 400µS/cm and pH = 8.3

All experiments in Figure 4-14 showed a trend of decreasing *E.coli* population with increasing fluence. However, experiments 1 and 3 both had initial growth occurring before the *E.coli* populations declined. Experiment 1 with the steepest slope achieved the highest inactivation with ~ 1.7 log. The other two experiments had similar linear approximations even though the data appeared different. Table 4-22 lists the inactivation constants along with the mean and SE.

Table 4-22: Results for Slope Coefficient for E.coli with SC of 400 µS/cm and pH = 8.3

	Mean	S.E.	Exp 1	Exp 2	Exp 3
k_1 (cm^2/kJ)	19	2	24	18	16
R^2	-	-	0.94	0.88	0.84
Skew	= 1.42 < 2.83 so data is positively skewed but not significantly				

The steep slope of experiment 1 from Table 4-22 skewed the data positively but not significantly with a value of 1.42. The large distribution in slope values caused the SE to be high at more than 10% of the mean.

4.3.5 pH = 8.3 and specific conductance of 700 µS/cm

No lag occurred in the three *E.coli* experiments with SC = 700 µS/cm and pH = 8.3. The results along with the linear approximations are shown in Figure 4-15.

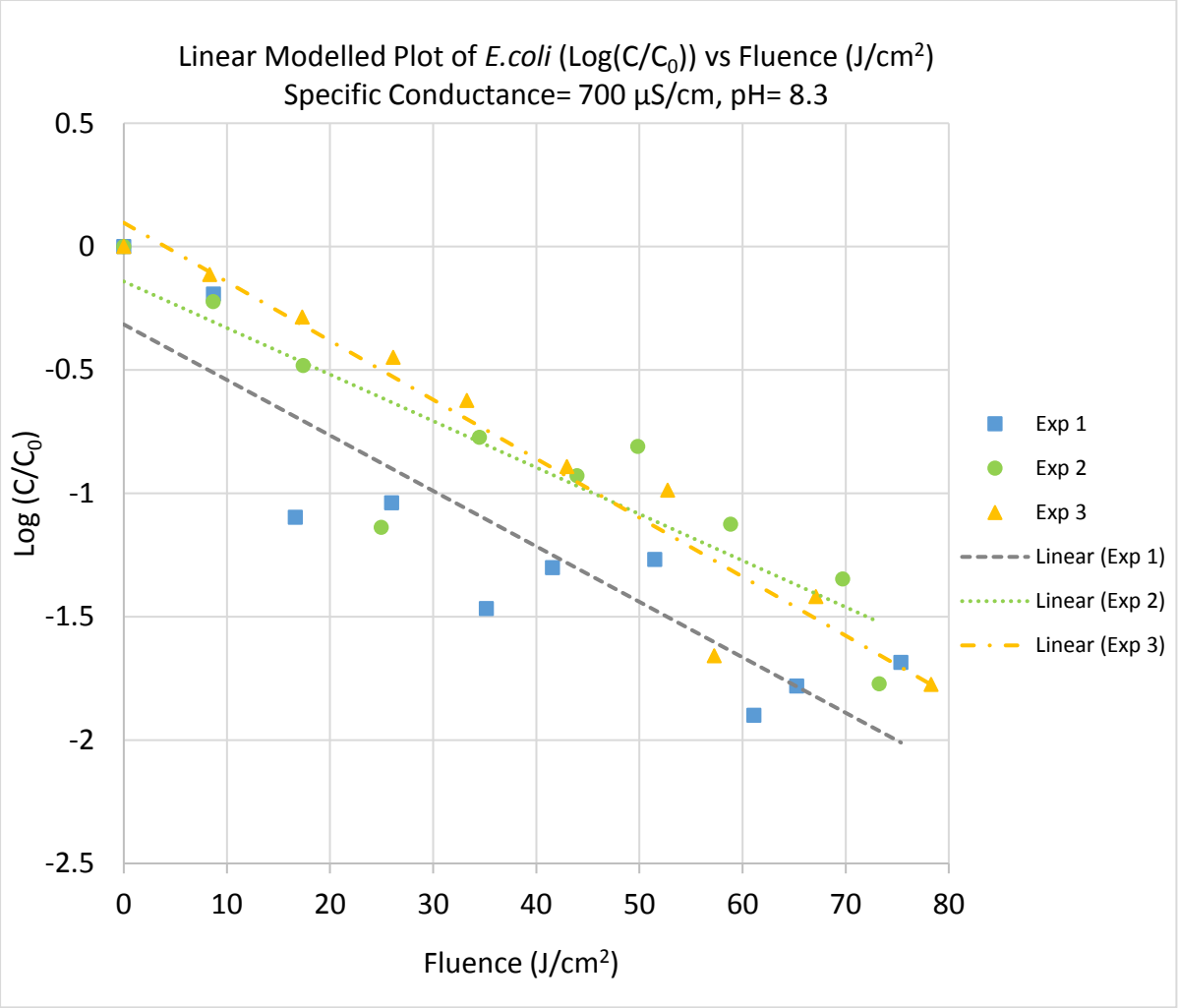

Figure 4-15: Linear Approximation of Plot of E.coli Reduction with Respect to Fluence for a Specific Conductance of 700µS/cm and pH = 8.3

All experiments shown in Figure 4-15 displayed a decrease in pathogen numbers with an increase in fluence. The linear approximations appeared to fit to the results for Experiments 2 and 3. Experiment 1 showed waves of inactivation followed by growth three times during the experiment. When this was modelled, the slope was very similar to the other experiments, as

indicated by a parallel line. The values for the mean, SE, R^2 and slopes are shown in Table 4-23.

Table 4-23: Results for slope coefficient for E.coli with SC of 700 µS/cm and pH = 8.3

	Mean	S.E.	Exp 1	Exp 2	Exp 3
k_1 (cm^2/kJ)	22	1	22	24	19
R^2	-	-	0.80	0.94	0.81
Skew	= -1.18 < 2.83 so data is negatively skewed but not significantly				

Although the data in Table 4-23 was negatively skewed with a value of -1.18, the skew was not statistically significant. The tight cluster of slope values minimised the SE to < 5% of the mean. Close approximations were made with the linear models with R^2 values 0.8 and above.

4.3.6 pH = 8.3 and specific conductance of 900 µS/cm

There were no obvious lag in the results from the three experiments with SC = 900 µS/cm and pH=8.3. The results were plotted along with linear approximations in Figure 4-16.

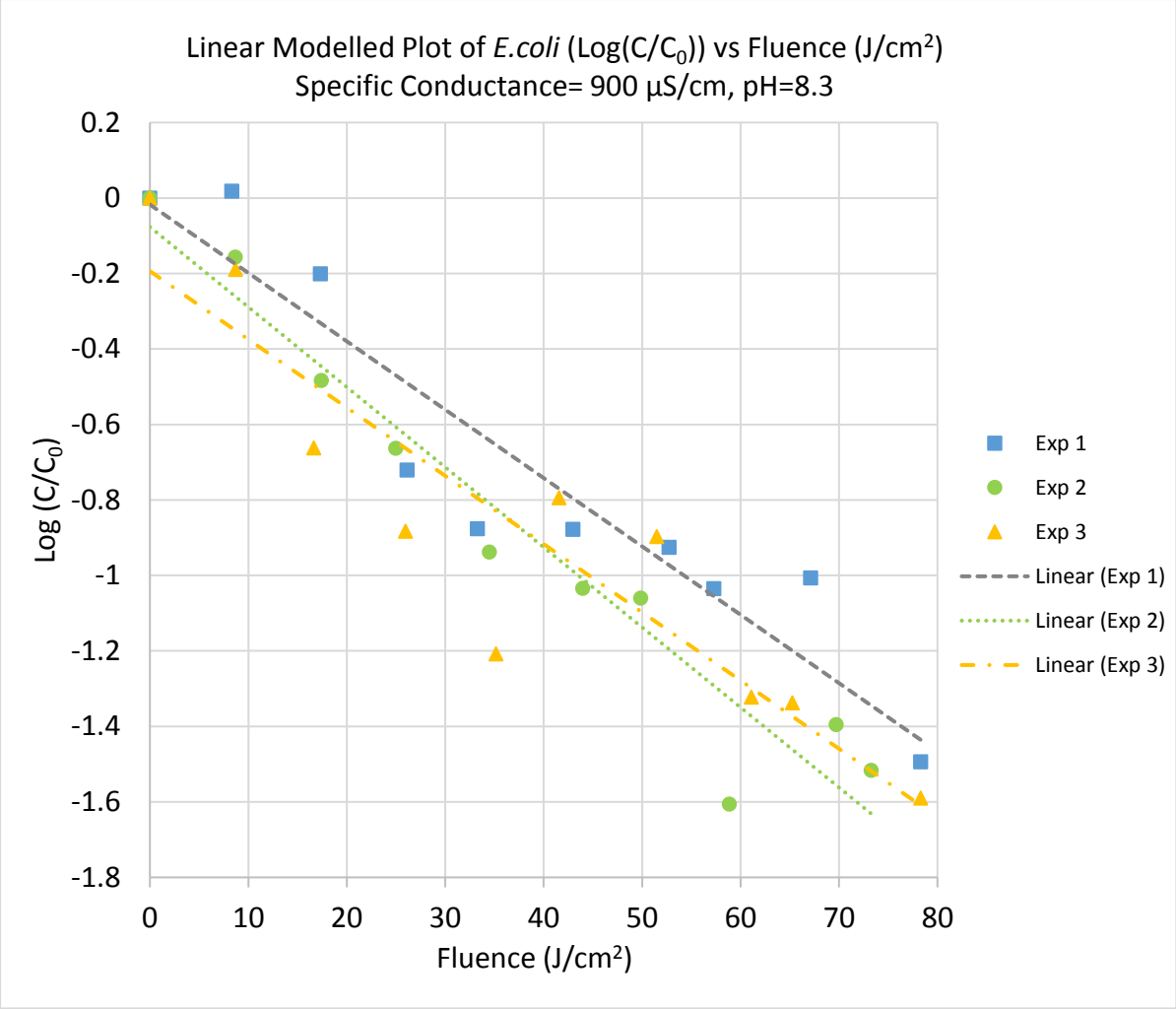

Figure 4-16: Linear Approximation of Plot of E.coli Reduction with Respect to Fluence for a Specific Conductance of 900µS/cm and pH = 8.3

All three experiments in Figure 4-16 followed the trend of reducing *E.coli* numbers for increasing fluence with the highest inactivation = 1.6 log (97.4%). Experiments 1 and 3 both had wide scatter patterns in the raw data. However, all the experiments had similar linear approximations. Table 4-24 lists the inactivation constants (k1) together with the mean and SE.

Table 4-24: Results for Slope Coefficient for E.coli with SC of 900 µS/cm and pH = 8.3

	Mean	S.E.	Exp 1	Exp 2	Exp 3
k_1 (cm^2/kJ)	19	1	18	21	18
R^2	-	-	0.89	0.94	0.84
Skew	= 1.73 < 2.83 so data is positively skewed but not significantly				

The data had a high non-statistically significant positive skew of 1.73 but a small SE of ~ 5% of the mean as shown in Table 4-24. High R^2 values indicated the model was a close fit to the data.

4.3.7 *E.coli* – water hardness

To measure the effect of water hardness on *E.coli* three experiments were run with a total hardness = 530mg/L $CaCO_3$, pH = 8.3 and a SC = 1830µS/cm. The doubling of the water conductivity from the previous experiments (400 – 900 µS/cm) is a direct result of increasing the total water hardness. This was not ideal as both the conductivity and total hardness increased over the previous experiments. The results for the experiments are shown in Figure 4-17 along with linear approximations of the data.

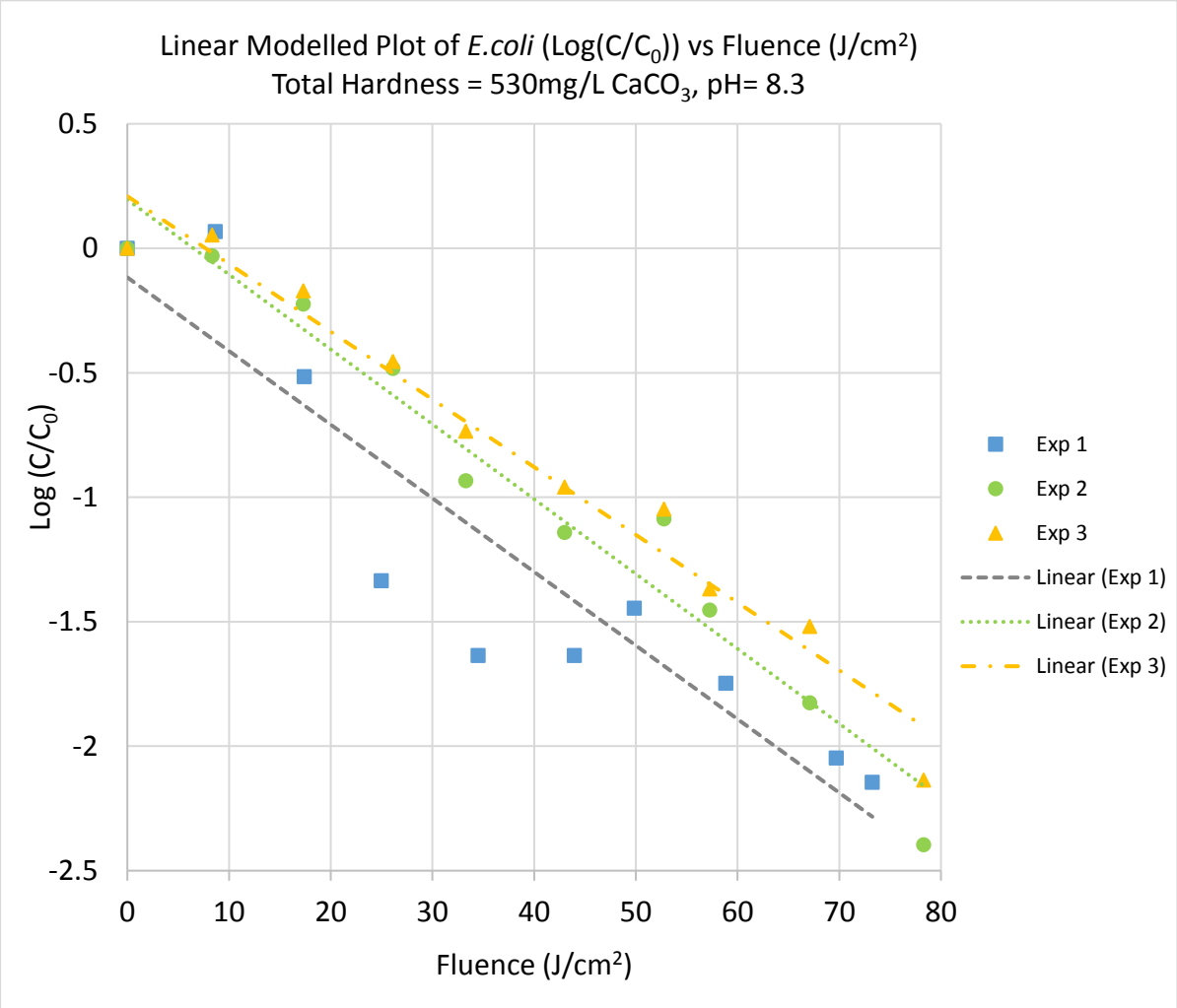

Figure 4-17: Linear Approximation of Plot of E.coli Reduction with Respect to Fluence for a Total Hardness = 530mg/L $CaCO_3$, Specific Conductance = 1830µS/cm and pH = 8.3

All experiments in Figure 4-17 followed the trend of reducing *E.coli* numbers with increasing fluence. The final inactivation was similar across all the experiments with a maximum of ~2.4

log occurring in experiment 2. There was a small lag experienced by all three experiments but it did not appear to affect the linear approximation of the results. The initial stages of experiment 1 had large variations in inactivation before becoming linear towards the end. The three experiments all had a similar slope to their respective modelled line. The inactivation constants are listed in Table 4-25 along with the mean, SE, skew and R^2 values.

Table 4-25: Results for Slope Coefficient for E.coli with Total Hardness = 530mg/L $CaCO_3$, SC of 1830 µS/cm, and pH = 8.3

	Mean	S.E.	Exp 1	Exp 2	Exp 3
k_1 (cm^2/kJ)	29	1	30	30	27
R^2	-	-	0.86	0.96	0.97
Skew	= -1.53 < 2.83 so data is negatively skewed but not significantly				

As two of the values for the slopes shown in Table 4-25 were quite close, the third value caused the distribution to be non-significantly negatively skewed with a skew = -1.53. However the overall close grouping minimised the SE to <5% of the mean. Excellent linear modelling was achieved in experiment 2 and 3 with R2 = 0.96 and 0.97 respectively.

4.3.8 *E.coli* – bottle size

The bottle size was reduced from the standard 93mm diameter bottles to smaller 60mm diameter bottles. This was to investigate the influence of water depth on the SODIS method. The results from the three experiments along with the linear approximations were plotted in Figure 4-18.

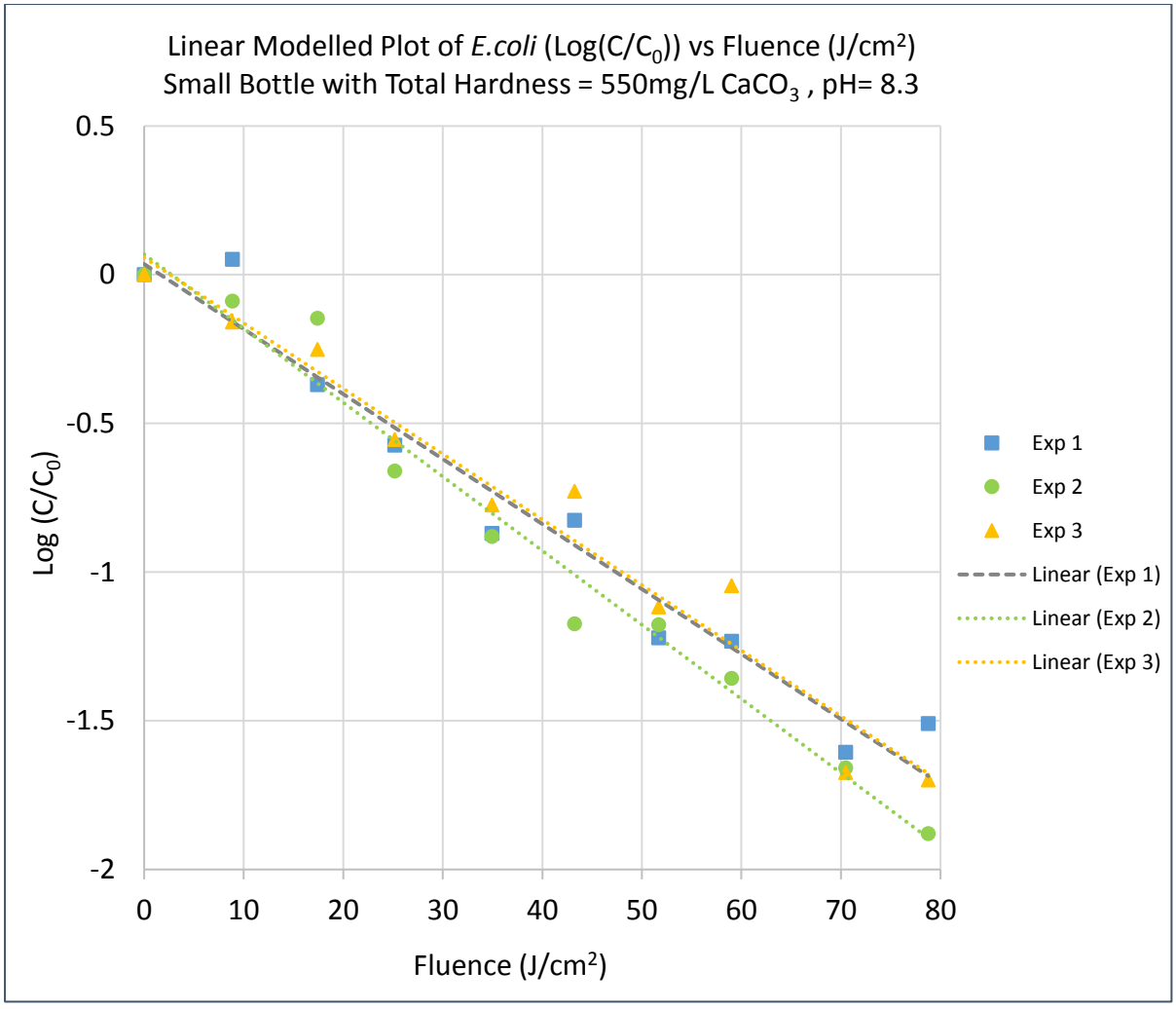

Figure 4-18: Linear Approximation of Plot of E.coli Reduction with Respect to Fluence for Small Bottles, Total Hardness =550mg/L $CaCO_3$, Specific Conductance = 1830µS/cm and pH = 8.3

The results trend of the data in Figure 4-18 was a reduction in *E.coli* population as fluence increases. All the experiments had close clusters of data points and the linear approximations appeared a good fit. The final inactivation ranged from 1.5 - 1.8 log, with similar slopes on all three linear approximations. The values of the slopes, mean and SE are listed in Table 4-26.

Table 4-26: Slope Coefficients for E.coli in Small Bottles with Total Hardness >550mg/L $CaCO_3$, SC = 1830µS/cm and pH = 8.3

	Mean	S.E.	Exp 1	Exp 2	Exp 3
k_1 (cm^2/kJ)	23	1	22	25	22
R^2	-	-	0.96	0.97	0.96
Skew	= 1.72 < 2.83 so data is positively skewed but not significantly				

In Table 4-26 slope values were clustered close which minimised the SE to < 5% of the mean. Excellent linear modelling of the data was achieved with R^2 values of 0.96 and above. There was a positive skew but this was not significant. Therefore, the data was treated as normally distributed.

4.3.9 *E.coli* overall discussion

A combination of all the inactivation results from the *E.coli* experimental data was plotted on a box and whisker diagram in Figure 4-19. The statistical significance of the different slopes determined being listed in Table 4-27.

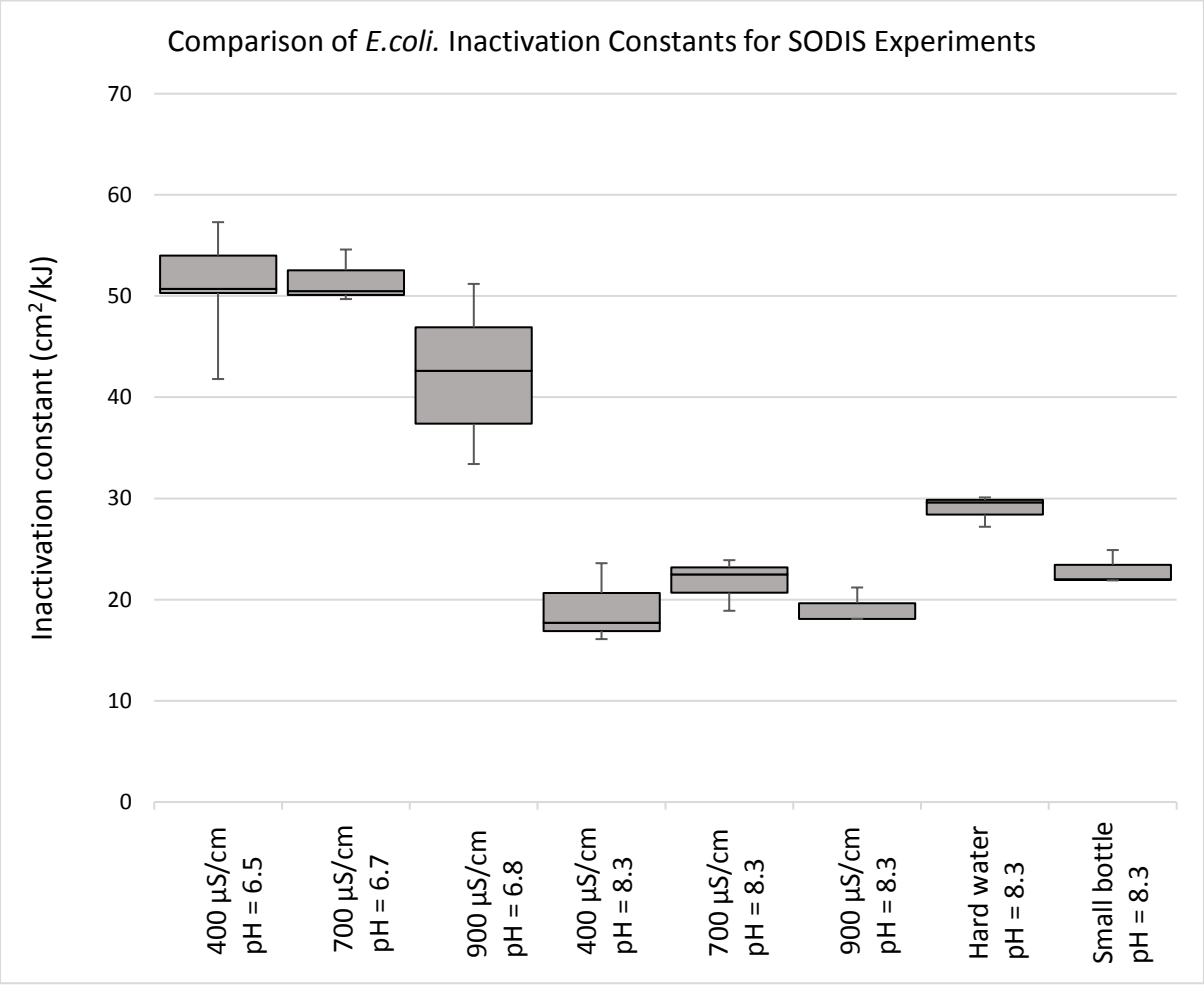

Figure 4-19: Box and Whisker Plot of E.coli Inactivation Constants (cm2/kJ) from all Experiments

Table 4-27: Statistical Significance of the Mean Slope for Various E.coli SODIS Experiments

(p = 0.05)	400µS/cm pH= 6.5	700µS/cm pH= 6.7	900µS/cm pH= 6.8	400µS/cm pH= 8.3	700µS/cm pH= 8.3	900µS/cm pH= 8.3	Hard 1830µS/cm pH= 8.3
700µS/cm pH= 6.7	0.709 = **NOT** Significant						
900µS/cm pH= 6.8	0.067 = **NOT** Significant	0.067 = **NOT** Significant					
400µS/cm pH= 8.3	<0.001 = Significant	<0.001 = Significant	0.004 = Significant				
700µS/cm pH= 8.3	<0.001 = Significant	<0.001 = Significant	0.005 = Significant	0.388 = **NOT** Significant			
900µS/cm pH= 8.3	<0.001 = Significant	<0.001 = Significant	0.003 = Significant	1 = **NOT** Significant	0.220 = **NOT** Significant		
Hard 1830µS/cm pH= 8.3	<0.001 = Significant	<0.001 = Significant	0.028 = Significant	0.016 = Significant	0.014 = Significant	0.002 = Significant	
Small bottle 1830µS/cm pH= 8.3	<0.001 = Significant	<0.001 = Significant	0.006 = Significant	0.2008 = **NOT** Significant	0.549 = **NOT** Significant	0.056 = **NOT** Significant	0.010 = Significant

There were two main groups shown on Figure 4-19, experiments with pH<7 and experiments with pH = 8.3.

pH < 7

All three sets of results for pH < 7 had to be lag corrected due to large lag periods occurring at the beginning of the experiments. From Table 4-27 it was identified there was no statistically significant difference between the inactivation rates for the conductivity experiments. However, the significance between SC = 900µS/cm and the other two experiments was only 0.067. This value is close to the p value of 0.05 used for determining significance. Running additional experiments would reduce the SE and possible indicate that there is a significant difference. The SODIS method should be designed for the most conservative conductivity i.e. for SC = 900µS/cm. This would ensure a minimum *E.coli* inactivation occurs under all conditions.

pH = 8.3

The lower inactivation rates for the pH= 8.3 experiments when compared to the pH < 7 experiments were in agreement with previously published research by Fisher et al. (2008) and Chong et al. (2011). They found that experiments with higher pH have lower inactivation rates. The three conductivity experiments with pH=8.3 all recorded the same inactivation coefficients, i.e. no statistically significant difference. An advantage of this is that it ensures a consistent method can be applied to cover the full conductivity range.

Hardness – Conductivity for pH=8.3

In Figure 4-19, the experiments with total water hardness = 530 mg/L $CaCO_3$ appear to have slightly higher inactivation than the low water hardness experiments for the same pH. Table 4-27 confirms the statistical significance of this higher inactivation. The cause could be attributed either to the increase in water hardness or to the increase in conductivity. As there was statistically no significant difference between the three conductivity experiments at pH = 8.3 it is unlikely that this increase in deactivation is through increased conductivity. Further investigation would be required to state that Hardness was responsible.

Bottle size

The standard 1.5L hardness experiments appeared to have a higher inactivation rate over the 355mL small bottle experiments. As these experiments both had hard water and high conductivity, the difference must be due to the different bottles used. Dessie et al. (2014) found that smaller diameter bottles had higher rates of inactivation in comparison to larger diameters. This was opposite to the results shown in Table 4-27, which indicated the higher inactivation rate for the larger bottles was statistically significant. A solution to this disagreement was that the PET used in the manufacture of the two bottles was characteristically different, with the large bottle having better UV transmission. This was investigated by recording the irradiance of the samples taken from the large and small bottles under a UV light of 0.547 mW/cm^2 irradiance. The UV transmission through the plastic samples recorded were 0.305 mW/cm^2 for the large bottle and 0.342 mW/cm^2 for the small bottle. This meant that the sample from the small bottle actually transmitted more light than the large bottle sample, so should have higher inactivation. This result added further confusion to why the inactivation of the small bottles was less than the larger bottles. Further investigation was carried out on the sample that was cut from the small bottle as shown in Figure 4-20.

Figure 4-20: Location of Sample Taken from Small PET bottle for UV Transmission Testing.

Figure 4-20 illustrates that the sample that was removed and tested was about one third of the bottle surface. When comparing the mass/volume (g/L) values for the small and large bottles from Table 4-16, the large bottles had a value of 27.2 g/L, which was less than half of the smaller bottles at 63.7g/L. Therefore, on average the plastic walls of the large bottle are considerably thinner than for the smaller bottle. The thickness of the plastic samples was determined with a digital micrometer (Mitutoyo) to be 0.251 ± 0.004mm and 0.288 ± 0.003mm for the large and small bottles respectively. Although the small bottle sample was thicker, it was not as thick as predicted with the mass/volume numbers. To balance this, the ends of the small bottle must be considerably thicker. This ultimately meant that the sample used to represent the smaller bottles was a poor representation of the bottle transmission test. Mani et al. (2006) designed a shallow but wide bottle specifically for use with the SODIS method. The performance improvements due to its shallower water depth were never realised due to an increase in thickness used for the bottle wall. Further testing is necessary to determine whether this is the case for the small bottle used.

4.4 Effect of bottle age on ultraviolet transmission

Samples were cut from the parallel middle section of the 1.5L PET bottles. When the different aged sections were laid adjacent to each other it was observed that they had different radii as shown in Figure 4-21.

Figure 4-21: Diameters of Samples Cut from 1.5L Sample Bottles; Youngest on Left to Oldest on Right.

Figure 4-21 shows from the left, two columns aged 150hrs, two columns aged 1000hrs, two columns aged 1500hrs and finally two columns aged 3000hrs. The 3000hr samples at 93mm were the same diameter as the uncut 93mm bottle. This relaxing of the residual stresses from production is likely caused from the UV lights. After the bottles had finished aging under the lights, they were kept in dark storage within the temperature-controlled room at 29°C adjacent to the ageing bottles. Therefore, the only variable is time under the UV lights.

In addition to the relaxing of the bottle diameter, the cut edge of the bottle samples reflected more light with more time under the lights. This can be seen in Figure 4-22 below which compares a 150hr sample with 3000hr sample.

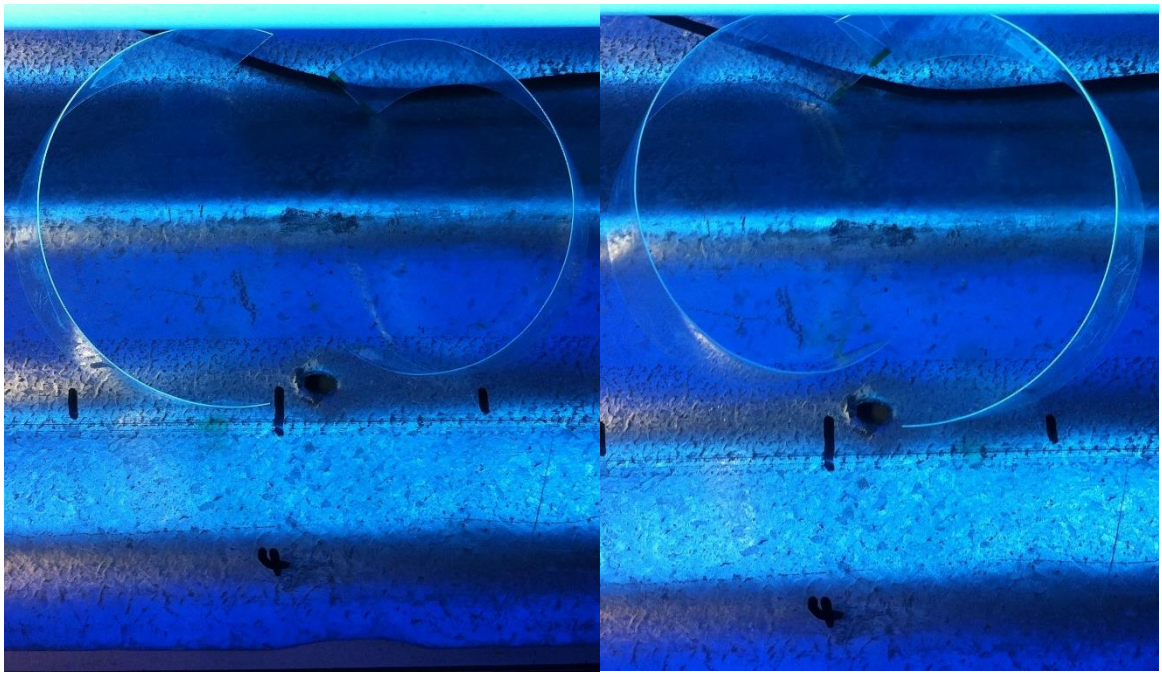

Figure 4-22: Comparison of Cut Edges for a)3000hr on Left- 150hr on Right b) 150hr on Left - 3000hr on Right

The samples were photographed before being swapped and photographed again as shown in Figure 4-22. In both a) and b) the cut edge of the 3000hr sample is brighter than the 150hr sample.

Table 4-28 shows the results from the aged bottle UV transmission experiments. The baseline irradiance recorded without any PET sample fitted was 0.549mW/cm^2.

Table 4-28: Results for Aged Bottles Experiments: Values of Irradiance are Mean ± Standard Error

Time under lights (hr)	No. of Observations	Irradiance (mW/cm^2) (% Reduction)	Skew / Significance level < 2.00
150	6	0.305 ± 0.004 (44%)	1.55 = **NOT** significantly skewed
1000	6	0.286 ± 0.007 (48%)	-0.28 = **NOT** significantly skewed
1500	6	0.247 ± 0.009 (55%)	0.26 = **NOT** significantly skewed
3000	6	0.218 ± 0.008 (60%)	-1.06 = **NOT** significantly skewed

The skew of the data groups from Table 4-28 changed from positively skewed to negatively skewed but none of the skew were statistically significant. Therefore, the results were treated as normally distributed. The results show a trend of decreasing irradiance with respect to time under the UV lights. This trend is clearer on the plot of irradiance vs time under UV lights in Figure 4-23.

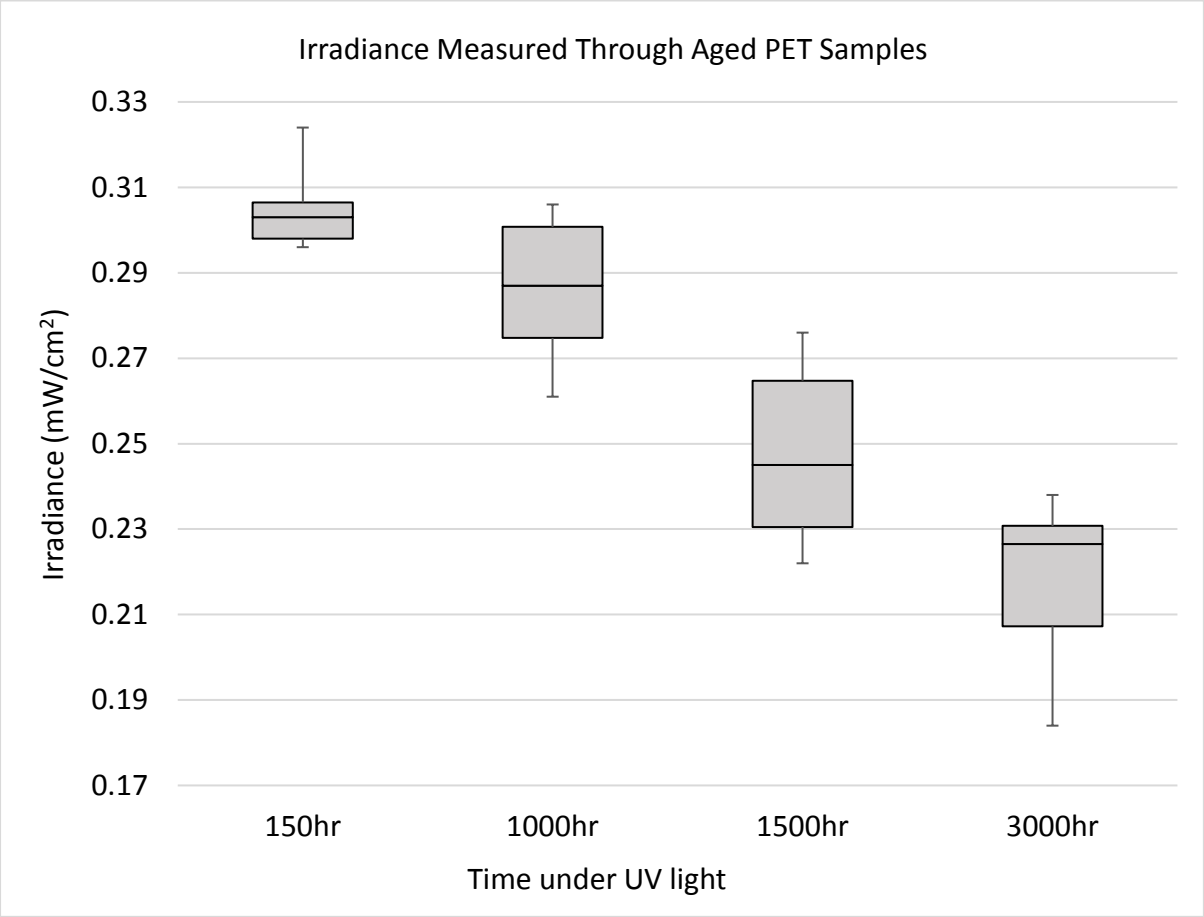

Figure 4-23: Results of aged bottle experiments - irradiance measured through aged pet samples

The clear trend in Figure 4-23 was a decrease in UV transmission with increase in time under the UV light. However even the youngest PET samples with 150hrs under the UV lights had a 45% reduction in UV transmission from the baseline UV light = 0.549 mW/cm^2. The UV transmission reduction from the baseline increased up to 60% for the 3000hr samples.

4.4.1 Statistical significance

The aged bottle UV transmission results were tested for significance using a two-tailed t-test with the results listed in Table 4-29.

Table 4-29: Results for Statistical Significance for Aged PET Samples using Two-Tailed T test with p = 0.05

P(T<=t)	150hrs	1000hrs	1500hrs
1000hrs	0.06 **NOT** Significant		
1500hrs	0.002 Significant	0.03 Significant	
3000hrs	<0.001 Significant	0.001 Significant	0.12 **NOT** Significant

The results from Table 4-29 clarify that there is no statistically significant difference between the UV transmission of the 150hr and the 1000hr samples; neither is there between the 1500hr sample and the 3000hr sample. The rest of the differences were statistically significant. Two possible reasons for the drop in UV transmission with age include, mechanical scratching and UV degradation.

Mechanical scratches occur when a bottle is placed onto a hard surface. While ageing the bottles, they were rotated one-quarter turn every day and cycled along the length of the UV light. A visual examination of the samples detected minimal scratches. Closer inspection of an uncut 1.5L bottle revealed that the shoulder and bottom of the bottle were wider than the sampled region. Consequently, they protected the sides of the bottle from scratches. Mechanical scratching of the PET can therefore be ruled out as the reason for the decrease in UV transmission.

The other reason for the reduced UV transmission is UV degradation. This was supported by Figure 4-22 which showed the cut edge of the longest aged bottle reflected more light. Figure 4-21 supported the degradation of the plastic with evidence of the change in diameter after prolonged UV exposure. No experiments were carried out investigating the effect of reduced UV irradiation on the SODIS method. To quantify the significance of the drop in UV transmission and establish a bottle replacement time, further research is required.

5 Conclusions and research recommendations

Recent population increases, cultural practices and climate change have increased the occurrences of diarrhoeal disease in Kiribati. With the link between polluted drinking water and diarrhoeal disease well established, appropriate methods for disinfecting the drinking water need to be explored (World Health Organization, 2010). The SODIS method was identified as a possible approach for Kiribati to meet current health outcome targets. Different water characteristics commonly found in Kiribati were investigated with respect to the SODIS method; the goal being to identify the impact of conductivity, pH, total water hardness, water depth i.e. bottle size and the age of bottle on the inactivation rates of total coliform and *E.coli.* Laboratory based experiments were used to approximate the conditions found in Kiribati. Linear modelling of the experimental results produced a good fit to the data and worked well with the small sample sizes.

5.1.1 Correlation of laboratory experiments to Kiribati

The water characteristics and the weather conditions of Kiribati were closely replicated in the laboratory experiments. Through using constant irradiance fluorescent UV lighting, locally sourced pathogens from Christchurch and simulated water there was no strong correlation to experiments carried out in Kiribati. Although there is no strong correlation externally, a strong correlation does exist between all the experiments performed.

5.1.2 Lag in results

There was a noticeable lag at the start of experiments carried out with a pH= 6.5 - 6.8. This was across all conductivity experiments for *E.coli*, but only noticeable at SC = 400µS/cm for the total coliform experiments. A lag period is the result of the DNA repair mechanism that exists in most living cells (Wegelin et al., 1994) .The primary effluent used as the source of the pathogens had a pH ~ 7. Therefore for the SODIS experiments at pH = 6.5 – 6.8 the pathogens were in their home environment. Increasing the pH to 8.3 could have pre-stressed the pathogens, activating their repair mechanism before the experiment started. This would result in an immediate response to the irradiation once the experiment began. The significance of this is that when investigating different pHs, the pathogens must be given sufficient time to adjust to the new pH conditions before the experiments are conducted else there is a risk of skewing the results.

5.1.3 Inactivation of total coliform versus *E.coli*

E.coli were more susceptible to UV inactivation than total coliform. Comparing the overall inactivation constants from the Box and Whisker plots in Figure 4-10 and Figure 4-19 confirmed this. For *E.coli,* the inactivation constants ranged from 15 - 50 cm^2/kJ whereas total coliform ranged from 8 – 25 cm^2/kJ. The significance of this finding is that treated water may be consumed under the incorrect assumption that it is safe based on *E.coli* inactivation. In reality other bacteria within the total coliform family from Figure 2-1 may be active and in significant numbers to cause disease or illness.

5.1.4 SODIS performance with respect to pH

Experiments with pH = 6.5 – 6.8 had higher rates of inactivation when compared to experiments with pH = 8.3 for the same SC. Experiments with pH = 6.5 and SC = 400µS/cm had the highest inactivation rates for both *E.coli* and total coliform with values of ~ 50 cm^2/kJ and 25 cm^2/kJ respectively. Conversely experiments with pH=8.3 and SC = 400µS/cm had the lowest inactivation rates for both *E.coli* and total coliform with values of ~ 15 cm^2/kJ and 8 cm^2/kJ respectively. The implications for Kiribati with its pH = 8.3 are that inactivation times that were established elsewhere based on lower pH experiments might not be applicable. To confirm this outcome, physical testing would need to be implemented in Kiribati.

5.1.5 Hard water

The difference in results from the experiments carried out with a total hardness > 530 mg/L $CaCO_3$ and those carried out with total hardness < 100mg/L $CaCO_3$ was mixed depending on pathogen type. For total coliform, there was no statistically significant difference in inactivation rates. For *E.coli,* the higher hardness experiments had higher inactivation constants. Theses higher inactivation constants for *E.coli* could be due either to the increase in hardness or the increase in conductivity. There was no statistically significant difference between soft water experiments for *E.coli* at different conductivity. Therefore, it is likely the difference in performance is from the increase in total water hardness. Kiribati has high water hardness so this finding may equate to a small improvement in SODIS performance. Additional experiments using a constant conductivity with increasing hardness would confirm if the increase in performance was due to the higher hardness.

5.1.6 Bottle size

It has been established that bottles with smaller diameters have a higher rate of inactivation over bottles with larger diameters (Dessie et al., 2014). Those results however are contrary to

the experimental results from this research. With *E.coli* the smaller bottles actually performed significantly worse than the larger diameter bottles. This does not disprove the original research; it merely highlights the error of assuming any small diameter bottle will always outperform a larger diameter bottle. When running experiments between bottles of different diameters or even different manufacturers, a full examination of the bottle including measuring the wall-thickness needs to be carried out. This is due to the strong influence the wall-thickness has on the overall pathogen inactivation. One of the advantages of the SODIS method is the cost, since that makes SODIS financially attractive amongst the poorer communities. Often these communities reuse bottles not originally designed for SODIS. With the significant influence wall-thickness has on pathogen inactivation, experiments in Kiribati need to be carried out with locally available bottles to establish their efficiency in deactivating pathogens for development of a local SODIS method.

5.1.7 Aged bottles – experiments with reduced irradiation

The experiments investigating the effect of artificially ageing bottles under the UV lights showed that statistically significant drops in UV transmission occur with time under lights. However, further research is required to correlate the drop in UV transmission to pathogen inactivation. This future research could be conducted in two ways. Firstly by using aged bottles in the experiments as this has the advantage of giving credible results. However, the disadvantage is that it requires substantial time at the beginning of the experiment to age the bottles. Secondly, by using new bottles and reducing the irradiance that is applied until it matched the values recorded from the aged bottle experiments, this allows for immediate investigation. The results however may not be as robust due to the relationship between irradiance and vertical distance Figure 3-2.

5.2 Overall conclusion

The predominant form of pathogen inactivation in this research was optical from the UV irradiation. This was identified from the sample bottle's temperature readings. That is, the maximum temperature reached by the 1.5L bottles was 36°C and 38°C for the small bottles. Both of these temperatures are less than 42 °C, which ensures optical inactivation is predominant. From the SODIS variables that were investigated: conductivity, pH, total hardness, water depth and aged bottles, some had a stronger influence on the pathogen inactivation. Based on their influence over pathogen inactivation from these experiments, the variables have been ranked from strongest (1) to weakest (4) in Table 5-1.

Table 5-1: Ranking of SODIS Variables Based on Their Influence Over Pathogen Inactivation During SODIS

Variable	Ranking	Justification
Bottle wall - thickness	1	Investigation of this variable was not carried out directly. However, during the small bottle experiments it acted against the positive influence of the reduced water depth to decrease the overall pathogen inactivation.
pH	2	Both pathogens exhibited statistically significant differences in pathogen inactivation with changes in pH. The rate at pH ~6.7 was approximately twice as fast as for pH =8.3.
Total hardness	3	Only *E.coli* responded to changes in total hardness. The inactivation improved with increased hardness.
Conductivity	4	Conductivity only significantly affected total coliforms at low pH, with the lowest rate being for SP = 700 µS/cm. With *E.coli*, there was no significant difference between the conductivity.
Aged bottle	n/a	A statistically significant difference in irradiance was determined. However, how this change in irradiance affects the pathogen inactivation is unknown. Further research is needed.
Bottle size	n/a	Due to the effect of the bottle wall-thickness, the effect of the bottle size could not be quantified. Further research is required.

5.3 Applicability of existing method to Kiribati.

Assuming equal irradiance with other published results, Kiribati will likely take longer to inactivate pathogens due to its high groundwater pH. Changes in conductivity at this high pH are not significant so any new method developed specifically for Kiribati would be applicable independent of rainfall. The high total water hardness would reduce the drop in pathogen inactivation; however, there would still be a net drop in performance due to the high pH.

5.4 Further research

The experimental results provided answers to how much influence the different variables have over the SODIS method. Due to the weak correlation between the experimental results and actual experiments carried out in Kiribati, further research is necessary to ensure that any new method developed meets or exceeds the inactivation of the current method. This would involve on site experiments using water and commonly used bottles sourced from the local area of interest. Prior to running the experiments, characterisation of the water should be carried out. The bare minimum must include pH and total hardness.

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Appendix A: Buffer solution and indicator used determining total hardness

Table A-1: Chemical makeup of buffer solution (on left) and indicator solution (on right)

Buffer solution	**Indicator**
16.4g Ammonium Chloride (NH_4Cl) 143mL Ammonia 1.179g EDTA 0.7g $MgSO_4.7H_2O$ to 250mL	Eriochrome Black T 0.5g Triethanol amine 100g

Appendix B: Results for pH = 6.5 and SC = 400µS/cm experiments

Experiment 1

Table A-1: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
6.52	406	-	-	-

Table A-2: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total. coliform MPN		E.coli	MPN	E.coli MPN		Temperature Light /Dark (°C)	
0	**500**	**49/27**	**517.2**	=	258600	**32/6**	**59.1**	=	29550	18.1	18.1
1	250	49/26	488.4	=	122100	32/3	53.8	=	13450	21.6	21.6
2	200	49/18	307.6	=	61520	28/2	42.6	=	8520	25.2	25.2
3	200	49/16	275.5	=	55100	30/7	55.4	=	11080	27.4	27.4
4	100	49/25	461.1	=	46110	31/7	58.1	=	5810	29	29
5	100	49/16	275.5	=	27550	20/2	27.5	=	2750	30.2	30.2
6	50	49/20	344.8	=	17240	26/0	35.5	=	1775	31.4	31.4
7	50	43/6	105	=	5250	7/1	8.5	=	425	32	32
8	20	49/20	344.8	=	6896	6/1	7.4	=	148	32.7	32.7
9	10	49/19	325.5	=	3255	13/0	14.8	=	148	33.1	33.1
Control	250	49/33	727	=	181750	31/5	36.8	=	9200		

Experiment 2

Table B-3: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
-	400	-	-	-

Table B-4: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total. coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**45/9**	**131.4**	=	65700	**18/1**	**23.1**	=	11550	18.1	18.1
1	250	49/16	275.5	=	68875	26/2	38.4	=	9600	21.6	18.6
2	200	49/15	261.3	=	52260	32/4	55.6	=	11120	25.2	19.8
3	200	46/7	133.3	=	26660	15/1	18.7	=	3740	27.4	21.4
4	200	41/8	98.7	=	19740	11/3	15.6	=	3120	29	22.8
5	100	46/9	142.1	=	14210	13/0	14.8	=	1480	30.2	23.5
6	50	49/20	344.8	=	17240	17/3	24.1	=	1205	31.4	24.3
7	50	38/4	74.9	=	3745	1/0	1	=	50	32	25.2
8	20	41/10	104.3	=	2086	2/0	2	=	40	32.7	25.7
9	10	48/13	201.4	=	2014	2/0	2	=	20	33.1	26
Control	250	49/26	488.4	=	122100	25/1	36.8	=	9200		

Experiment 3

Table B-5: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
-	398	-	-	-

Table B-6: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total. coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**49/17**	**290.9**	=	145450	**20/4**	**30.1**	=	15050	18.2	18.2
1	250	49/20	344.8	=	86200	30/3	48.7	=	12175	21.5	18
2	200	48/18	248.9	=	49780	25/4	39.3	=	7860	24.4	19.5
3	200	48/17	238.2	=	47640	24/2	34.5	=	6900	26.9	20.9
4	200	43/10	117.8	=	23560	23/1	31.3	=	6260	28.5	22
5	100	48/7	159.7	=	15970	26/1	36.9	=	3690	30.2	23.2
6	50	47/13	178.5	=	8925	18/3	25.6	=	1280	31.4	24.2
7	50	44/8	118.7	=	5935	12/0	13.5	=	675	32.2	24.7
8	20	45/8	127.4	=	2548	14/0	16.1	=	322	33.1	25.2
9	10	34/8	68.9	=	689	0/0	<1	<	10	32.2	25.6
Control	250	49/25	461.1	=	115275	28/2	42.6	=	10650		

Experiment 4

Table B-7: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
-	402	-	-	-

Table B-8: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total. coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**46/17**	**184.2**	=	92100	**14/2**	**18.5**	=	9250	16.6	16.6
1	250	48/18	248.9	=	62225	28/3	44.1	=	11025	20.7	16.9
2	200	48/13	201.4	=	40280	20/5	31.5	=	6300	25	19
3	200	49/7	179.3	=	35860	21/2	29.2	=	5840	27.5	20.9
4	200	38/8	83.9	=	16780	12/2	15.8	=	3160	28.5	21.9
5	100	38/8	83.9	=	8390	15/1	18.7	=	1870	30.4	23.2
6	50	47/10	160.7	=	8035	14/2	18.5	=	925	31.2	24.1
7	50	22/3	32.3	=	1615	6/0	6.3	=	315	32.2	24.9
8	20	37/7	77.6	=	1552	3/0	3.1	=	62	33.2	25.5
9	10	45/5	116.2	=	1162	1/0	1	=	10	33.9	26.3
Control	250	49/18	307.6	=	76900	26/0	35.5	=	8875		

Experiment 5

Table B-9: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
-	398	-	-	-

Table B-10: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total. coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**44/3**	**102.2**	=	51100	**17/0**	**20.3**	=	10150	16.6	16.6
1	250	47/9	155.3	=	38825	29/5	49.6	=	12400	20.7	16.9
2	200	48/15	218.7	=	43740	31/7	58.1	=	11620	25	19
3	200	46/13	161.6	=	32320	29/2	44.8	=	8960	27.5	20.9
4	200	43/6	105	=	21000	16/2	21.3	=	4260	28.5	21.9
5	100	43/6	105	=	10500	20/2	27.5	=	2750	30.4	23.2
6	50	42/6	98.8	=	4940	8/0	8.6	=	430	31.2	24.1
7	50	21/2	29.2	=	1460	3/1	4.1	=	205	32.2	24.9
8	20	48/14	209.8	=	4196	9/0	9.8	=	196	33.2	25.5
9	10	25/1	35	=	350	0/0	<1	<	10	33.9	26.3
Control	250	49/19	325.5	=	81375	35/3	62.4	=	15600		

Appendix C: Results for pH = 6.7 and SC = 700µS/cm experiments

Experiment 1

Table C-1: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
6.6	703	-	0.3	456

Table C-2: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**49/15**	**261.3**	=	130650	**25/2**	**36.4**	=	18200	20.1	20.1
1	250	49/20	344.8	=	86200	30/2	47.1	=	11775	25.3	22.6
2	200	49/27	517.2	=	103440	29/7	52.8	=	10560	27.8	23.6
3	200	49/21	365.4	=	73080	35/2	60.5	=	12100	30.6	25.1
4	200	48/14	209.8	=	41960	25/1	35	=	7000	31.6	25.9
5	100	49/15	261.3	=	26130	29/5	49.6	=	4960	32	25.7
6	50	49/34	770.1	=	38505	29/5	49.6	=	2480	33.9	27
7	25	49/24	435.2	=	10880	16/3	22.6	=	565	34.3	27.6
8	20	49/25	461.1	=	9222	13/2	17.1	=	342	35.3	28.4
9	10	49/33	727	=	7270	11/0	12.2	=	122	36	29.5
Control	250	49/31	648.8	=	162200	29/4	48	=	12000		

Experiment 2

Table C-3: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
6.39	697	-	0.3	453

Table C-4: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**49/11**	**214.3**	=	107150	**20/2**	**27.5**	=	13750	18.8	18.8
1	250	49/25	461.1	=	115275	25/2	36.4	=	9100	23.4	20
2	200	48/17	238.2	=	47640	32/4	55.6	=	11120	27.2	22.8
3	200	48/15	218.7	=	43740	36/3	65.7	=	13140	30.4	24.8
4	200	49/9	195.6	=	39120	25/5	40.8	=	8160	32	25.8
5	100	49/12	224.7	=	22470	26/1	36.9	=	3690	33.3	26.9
6	50	49/23	410.6	=	20530	34/6	65	=	3250	33.9	27.5
7	25	49/24	435.2	=	10880	34/1	55.7	=	1392.5	35.6	28.6
8	20	49/27	517.2	=	10344	15/2	19.9	=	398	35.4	28.9
9	10	49/25	461.1	=	4611	16/1	20.1	=	201	36.5	28.9
Control	250	49/37	920.8	=	230200	31/3	51.2	=	12800		

Experiment 3

Table C-5: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
6.6	701	-	0.3	456

Table C-6: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**48/11**	**186**	=	93000	**18/1**	**35.9**	=	17950	20.8	20.8
1	250	49/21	365.4	=	91350	31/5	54.6	=	13650	25.9	23.2
2	200	49/18	307.6	=	61520	36/3	65.7	=	13140	28.9	24.5
3	200	49/16	275.5	=	55100	33/1	53	=	10600	30.6	25.5
4	200	49/18	307.6	=	61520	34/6	65	=	13000	32.8	26.6
5	100	49/24	435.2	=	43520	35/7	70.3	=	7030	33.9	27.4
6	50	49/27	517.2	=	25860	38/7	81.6	=	4080	34.2	28.2
7	25	49/35	816.4	=	20410	37/4	71.2	=	1780	36.2	28.7
8	20	49/32	686.7	=	13734	19/6	31.1	=	622	36.7	29.4
9	10	49/27	517.2	=	5172	16/1	20.1	=	201	36.4	30.2
Control	250	49/27	517.2	=	129300	34/4	61.3	=	15325		

Experiment 1

Table D-1: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
-	901	-	0.4	586

Table D-2: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**47/6**	**140.1**	=	70050	**23/3**	**34.1**	=	17050	16.2	16.2
1	250	48/18	248.9	=	62225	36/4	67.7	=	16925	22.4	18.8
2	200	49/10	204.6	=	40920	36/2	63.7	=	12740	26	20.5
3	200	43/12	124.6	=	24920	27/2	40.4	=	8080	28.3	21.7
4	200	44/6	111.9	=	22380	19/3	27.2	=	5440	29.7	22.8
5	100	44/8	118.7	=	11870	12/0	13.5	=	1350	31.4	24
6	50	46/11	151.5	=	7575	8/0	8.6	=	430	32.1	24.9
7	50	33/4	58.3	=	2915	0/0	<1	<	50	33.4	25.4
8	20	33/9	67.6	=	1352	2/0	2	=	40	33.8	26
9	10	47/12	172.3	=	1723	5/0	5.2	=	52	33.3	26.5
Control	250	49/24	435.2	=	108800	31/6	56.3	=	14075		

Experiment 2

Table D-3: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
-	897	-	-	-

Table D-4: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**46/16**	**178.2**	=	89100	**27/1**	**38.9**	=	19450	17.9	17.9
1	250	48/13	201.4	=	50350	29/2	44.8	=	11200	21.5	18
2	200	49/18	307.6	=	61520	35/8	72.3	=	14460	25.2	19.8
3	200	46/13	161.6	=	32320	31/4	52.9	=	10580	27.9	21.2
4	200	40/5	85.7	=	17140	13/2	17.1	=	3420	29.2	22.6
5	100	43/13	128.1	=	12810	10/1	12.1	=	1210	30.9	23.6
6	50	47/4	130.9	=	6545	8/0	8.6	=	430	32.2	24.5
7	25	49/10	204.6	=	5115	5/0	5.2	=	130	32.8	25.2
8	20	49/20	344.8	=	6896	4/0	4.1	=	82	33.9	25.7
9	10	49/22	365.4	=	3654	5/0	5.2	=	52	34.2	26.3
Control	250	49/33	727	=	181750	35/2	60.5	=	15125		

Experiment 3

Table D-5: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
6.8	910		-	-

Table D-6: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**49/15**	**261.3**	=	130650	**15/1**	**18.7**	=	9350	19.8	19.8
1	250	49/18	307.6	=	76900	27/4	43.5	=	10875	27	24
2	200	49/27	517.2	=	103440	33/1	53	=	10600	29.5	25
3	200	49/20	344.8	=	68960	25/2	36.4	=	7280	28.8	23.3
4	200	49/13	235.9	=	47180	20/2	27.5	=	5500	32.2	26.1
5	100	49/18	307.6	=	30760	21/2	29.2	=	2920	33.4	27.5
6	50	49/13	235.9	=	11795	22/1	29.5	=	1475	34.5	28.2
7	25	49/18	307.6	=	7690	7/3	10.7	=	267.5	36	29.2
8	20	49/16	275.5	=	5510	7/1	8.5	=	170	35.6	29.3
9	10	49/25	461.1	=	4611	8/0	8.6	=	86	36.4	29.8
Control	250	49/34	770.1	=	192525	34/5	63.1	=	15775		

Experiment 4

Table D-7: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
6.73	926	-	0.5	602

Table D-8: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**49/25**	**461.1**	=	230550	**18/1**	**23.1**	=	11550	19	19
1	250	49/21	365.4	=	91350	22/1	30.9	=	7725	24.5	21.4
2	200	49/14	248.1	=	49620	24/5	38.8	=	7760	27.5	22.3
3	200	48/9	172.2	=	34440	21/0	26.5	=	5300	29.9	24.3
4	200	48/9	172.2	=	34440	13/3	18.3	=	3660	31.7	25.6
5	100	49/18	307.6	=	30760	12/3	16.9	=	1690	33.1	26.2
6	50	48/13	201.4	=	10070	8/1	9.7	=	485	34	27.1
7	25	49/30	613.1	=	15327.5	14/1	17.3	=	432.5	35.4	27.9
8	20	49/23	410.6	=	8212	8/1	9.7	=	194	36	28.7
9	10	49/19	325.5	=	3255	9/0	9.8	=	98	35.9	29.2
Control	250	49/40	1119.9	=	279975	26/5	42.8	=	10700		

Experiment 1

Table E-1: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
8.3	404	40	0.2	263

Table E-2: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	500	47/9	155.3	=	77650	22/2	30.9	=	15450	20.3	20.3
1	250	49/23	410.6	=	102650	41/7	95.9	=	23975	25.8	23
2	200	49/14	248.1	=	49620	28/2	42.6	=	8520	28.4	24
3	200	45/12	143.9	=	28780	12/1	14.6	=	2920	30.7	25.1
4	200	45/9	131.4	=	26280	10/2	13.2	=	2640	32.7	26.1
5	100	48/20	272.3	=	27230	13/1	16	=	1600	33.3	27
6	100	49/20	344.8	=	34480	14/3	19.7	=	1970	34.4	27.7
7	50	49/22	387.3	=	19365	12/0	13.5	=	675	35.2	28.2
8	25	49/33	727	=	18175	17/3	24.1	=	602.5	35.3	28.8
9	20	49/32	686.7	=	13734	13/1	16	=	320		

Experiment 2

Table E-3: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
8.3	403	36	0.2	262

Table E-4: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN		Total coliform MPN	*E.coli*	MPN		*E.coli* MPN	Temperature Light /Dark (°C)	
0	500	49/15	261.3	=	130650	27/4	43.5	=	21750	21.2	21.2
1	250	49/32	686.7	=	171675	35/3	62.4	=	15600	26.7	23.8
2	200	49/20	344.8	=	68960	31/5	54.6	=	10920	29.3	25.1
3	200	49/22	387.3	=	77460	13/1	16	=	3200	31.5	26.1
4	200	49/18	307.6	=	61520	26/2	38.4	=	7680	32.5	27
5	100	49/29	579.4	=	57940	26/3	39.9	=	3990	33.5	27.7
6	100	49/33	727	=	72700	26/3	39.9	=	3990	34.4	28.3
7	50	49/44	1553.1	=	77655	24/4	37.3	=	1865	34.3	28.8
8	25	49/46	1986.3	=	49657.5	37/3	69.1	=	1727.5	35.3	29.1
9	20	49/43	1413.6	=	28272	21/3	30.5	=	610		

Experiment 3

Table E-5: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
8.3	401	36	0.2	261

Table E-6: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN		Total coliform MPN	*E.coli*	MPN		*E.coli* MPN	Temperature Light /Dark (°C)	
0	500	49/10	204.6	=	102300	19/2	24.6	=	12300	20.6	20.6
1	250	49/25	461.1	=	115275	34/3	59.4	=	14850	26.4	24.2
2	200	49/17	290.9	=	58180	14/0	16.1	=	3220	30	26.1
3	200	49/18	307.6	=	61520	9/0	9.8	=	1960	31.5	27
4	200	49/15	261.3	=	52260	11/1	13.4	=	2680	33.1	28
5	200	47/18	214.2	=	42840	6/1	7.4	=	1480	34.8	28.8
6	100	49/26	488.4	=	48840	11/4	16.8	=	1680	35.1	29.3
7	50	49/33	727	=	36350	21/4	31.8	=	1590	35.9	29.8
8	25	49/43	1413.6	=	35340	26/8	47.4	=	1185	36.3	30.2
9	20	49/42	1299.7	=	25994	19/0	23.3	=	466		

Appendix F: Results for pH = 8.3 and SC = 700µS/cm experiments

Experiment 1

Table F-1: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
8.3	703	~62	0.3	457

Table F-2: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**49/20**	**344.8**	=	172400	**29/4**	**48**	=	24000	20.9	20.9
1	250	49/25	461.1	=	115275	36/1	61.7	=	15425	26.6	24.4
2	200	47/9	155.3	=	31060	7/2	9.6	=	1920	29	25.4
3	200	45/8	127.4	=	25480	10/0	11	=	2200	31.4	26.2
4	200	46/9	142.1	=	28420	4/0	4.1	=	820	32.5	27
5	100	49/27	517.5	=	51750	9/2	12	=	1200	33.4	27.8
6	50	49/26	488.4	=	24420	19/2	25.9	=	1295	34.4	28.3
7	25	49/27	517.5	=	12937.5	10/1	12.1	=	302.5	34.8	28.9
8	20	49/39	1046.2	=	20924	15/2	19.9	=	398	35.8	29.5
9	10	49/46	1986.3	=	19863	29/5	49.6	=	496	35.9	30.1
Control	250	49/37	920.8	=	230200	37/2	67	=	16750		

Experiment 2

Table F-3: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
8.3	706	~62	0.3	459

Table F-4: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**49/24**	**435.2**	=	217600	**23/3**	**34.1**	=	17050	20.8	20.8
1	250	48/27	378.4	=	94600	25/5	40.8	=	10200	25.9	22.6
2	200	49/24	435.2	=	87040	18/5	28.1	=	5620	28.8	24.6
3	200	49/21	365.4	=	73080	4/2	6.2	=	1240	31	25.7
4	200	49/21	365.4	=	73080	10/3	14.4	=	2880	33	26.8
5	100	49/25	461.1	=	46110	16/1	20.1	=	2010	33.4	27.2
6	50	49/39	1046.2	=	52310	31/4	52.9	=	2645	34.3	28.2
7	25	49/41	1203.3	=	30082.5	31/3	51.2	=	1280	35.6	28.6
8	20	49/46	1986.3	=	39726	23/6	38.3	=	766	35.7	29.2
9	10	49/36	866.4	=	8664	20/3	28.8	=	288	36	29.4
Control	250	49/28	547.5	=	136875	29/3	46.4	=	11600		

Experiment 3

Table F-5: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
8.3	704	~62	0.3	458

Table F-6: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**49/11**	**214.3**	=	107150	**23/6**	**38.3**	=	19150	20.6	20.6
1	250	49/23	410.6	=	102650	32/6	59.1	=	14775	26.1	23.1
2	200	49/23	410.6	=	82120	29/5	49.6	=	9920	28.9	24
3	200	49/19	325.5	=	65100	23/3	34.1	=	6820	30.9	25.5
4	200	49/19	325.5	=	65100	17/2	22.8	=	4560	32	26.2
5	100	49/27	517.2	=	51720	19/1	24.6	=	2460	33.8	27.4
6	100	49/24	435.2	=	43520	14/3	19.7	=	1970	34.3	28
7	50	49/14	248.1	=	12405	6/2	8.4	=	420	35.4	28.4
8	25	49/39	1046.2	=	26155	21/2	29.2	=	730	36.1	29
9	20	49/29	579.4	=	11588	14/0	16.1	=	322	36.1	29.8
Control	250	49/27	517.2	=	129300	34/7	67	=	16750		

Experiment 1

Table G-1: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
8.3	911	80	0.4	592

Table G-2: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**49/25**	**461.1**	=	230550	**25/2**	**36.4**	=	18200	20.8	20.8
1	250	49/30	613.1	=	153275	36/8	75.9	=	18975	22.7	20.1
2	200	49/19	325.5	=	65100	32/5	57.3	=	11460	28.9	24.5
3	200	49/17	290.9	=	58180	14/1	17.3	=	3460	30.4	25.8
4	200	49/19	325.5	=	65100	10/1	12.1	=	2420	32.3	26.8
5	100	49/31	648.8	=	64880	17/3	24.1	=	2410	33.4	27.5
6	100	48/24	328.2	=	32820	17/1	21.6	=	2160	34	28.2
7	50	49/40	1119.9	=	55995	22/4	33.6	=	1680	35.3	28.5
8	25	49/39	1046.2	=	26155	36/6	71.7	=	1792.5	35.7	29.1
9	20	49/40	1119.9	=	22398	21/2	29.2	=	584		

Experiment 2

Table G-3: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
8.3	907	78	0.4	590

Table G-4: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**49/17**	**290.9**	=	145450	**27/3**	**42**	=	21000	20.8	20.8
1	250	49/18	325.5	=	81375	35/1	58.6	=	14650	25.2	22.8
2	200	48/20	272.3	=	54460	24/2	34.5	=	6900	28.3	24.3
3	200	48/19	260.3	=	52060	17/2	22.8	=	4560	30.9	25.5
4	200	49/19	325.5	=	65100	10/1	12.1	=	2420	32.6	26.6
5	200	45/10	135.4	=	27080	8/1	9.7	=	1940	33.6	27.5
6	100	49/19	325.5	=	32550	13/3	18.3	=	1830	34.1	28.2
7	100	48/24	328.2	=	32820	5/0	5.2	=	520	35	28.8
8	50	49/31	648.8	=	32440	12/3	16.9	=	845	35.2	29.3
9	25	49/39	1046.2	=	26155	18/3	25.6	=	640		

Experiment 3

Table G-5: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
8.3	905	78	0.4	588

Table G-6: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**49/25**	**461.1**	=	230550	**22/4**	**33.6**	=	16800	19.9	19.9
1	250	49/19	325.5	=	81375	27/4	43.5	=	10875	25.4	22.8
2	200	49/17	290.9	=	58180	13/3	18.3	=	3660	28.8	24.3
3	200	48/20	272.3	=	54460	10/0	11	=	2200	30.9	25.4
4	200	44/8	118.7	=	23740	5/0	5.2	=	1040	32.1	26.7
5	200	48/12	193.5	=	38700	12/0	13.5	=	2700	33	27.2
6	100	48/12	193.5	=	19350	16/2	21.3	=	2130	34.9	28.2
7	50	49/19	325.5	=	16275	13/1	16	=	800	34.4	28.6
8	25	49/29	579.4	=	14485	22/2	30.9	=	772.5	35.4	28.9
9	20	49/27	517.2	=	10344	17/1	21.6	=	432		

Appendix H: Results for total hardness >500mg/L $CaCO_3$ experiments

Experiment 1

Table H-1: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
8.3	~1830	524	0.3	454

Table H-2: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN		Total coliform MPN	*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**48/15**	**218.7**	=	109350	**14/1**	**17.3**	=	8650	20.8	20.8
1	250	48/13	201.4	=	50350	27/2	40.4	=	10100	26.2	22.9
2	200	41/8	98.7	=	19740	10/2	13.2	=	2640	28.8	24.8
3	200	32/4	55.6	=	11120	1/1	2	=	400	31.2	25.8
4	200	22/3	32.3	=	6460	1/0	1	=	200	33	26.9
5	100	38/9	86.2	=	8620	2/0	2	=	200	33.4	27.5
6	100	39/4	78.9	=	7890	3/0	3.1	=	310	34.5	28.3
7	50	37/4	71.2	=	3560	3/0	3.1	=	155	35.5	28.7
8	25	46/8	137.6	=	3440	3/0	3.1	=	77.5	35.1	29.4
9	20	44/13	137.4	=	2748	3/0	3.1	=	62		

Experiment 2

Table H-3: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
8.3	~1830	544	0.3	454

Table H-4: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**49/26**	**488.4**	=	244200	**26/5**	**42.8**	=	21400	21.1	21.1
1	250	49/30	613.1	=	153275	37/8	79.8	=	19950	26.2	23.4
2	200	49/20	344.8	=	68960	33/7	63.8	=	12760	29.4	25.1
3	100	49/25	461.1	=	46110	35/7	70.3	=	7030	30.9	25.9
4	100	49/25	461.1	=	46110	20/0	24.9	=	2490	32.6	27
5	50	49/29	579.4	=	28970	22/2	30.9	=	1545	33.8	27.8
6	50	49/31	648.8	=	32440	22/5	35	=	1750	34.3	28.5
7	25	49/34	770.1	=	19252.5	20/4	30.1	=	752.5	35.2	28.6
8	20	49/37	920.8	=	18416	13/1	16	=	320	35.5	29.2
9	10	49/42	1299.7	=	12997	8/0	8.6	=	86		

Experiment 3

Table H-5: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
8.3	~1830	528	0.3	454

Table H-6: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**49/23**	**410.6**	=	205300	**24/2**	**34.5**	=	17250	21.1	21.1
1	250	49/31	648.8	=	162200	36/9	78	=	19500	26.4	23
2	200	49/22	387.3	=	77460	31/7	58.1	=	11620	29.1	24.6
3	100	49/22	387.3	=	38730	35/2	60.5	=	6050	31.4	25.7
4	100	49/24	435.2	=	43520	21/4	31.8	=	3180	32.4	26.6
5	50	49/25	461.1	=	23055	25/3	37.9	=	1895	33.6	27.5
6	50	49/27	517.2	=	25860	22/2	30.9	=	1545	34.8	28
7	25	49/26	488.4	=	12210	22/1	29.5	=	737.5	34.8	28.6
8	25	49/30	613.1	=	15327.5	14/4	20.9	=	522.5	35.6	29.2
9	20	49/22	387.3	=	7746	6/0	6.3	=	126		

Experiment 1

Table I-1: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
8.3	~1830	564	0.3	456

Table I-2: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**49/14**	**248.1**	=	124050	**22/3**	**32.3**	=	16150	20.2	20.2
1	250	49/21	365.4	=	91350	38/3	72.7	=	18175	27.9	24
2	200	49/28	547.5	=	109500	21/6	34.5	=	6900	32.8	26.3
3	200	48/15	218.7	=	43740	17/1	21.6	=	4320	34.8	27.9
4	200	48/10	178.9	=	35780	9/1	10.9	=	2180	36.3	28.8
5	100	48/13	201.4	=	20140	17/3	24.1	=	2410	36.2	29.5
6	100	47/14	185	=	18500	8/1	9.7	=	970	37.7	30.3
7	50	49/20	344.8	=	17240	16/0	18.9	=	945	38.3	30.6
8	25	49/26	488.4	=	12210	13/1	16	=	400	38	30.9
9	20	49/26	488.4	=	9768	16/5	25	=	500	37.6	30.8

Experiment 2

Table I-3: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
8.3	1829	552	0.3	458

Table I-4: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**49/21**	**365.4**	=	182700	**25/2**	**36.4**	=	18200	21	21
1	250	49/25	461.1	=	115275	34/3	59.4	=	14850	28.6	25.5
2	200	49/18	307.6	=	61520	34/6	65	=	13000	33.2	27
3	200	47/14	185	=	37000	15/2	19.9	=	3980	35.1	28.5
4	200	47/11	166.4	=	33280	9/2	12	=	2400	36.3	29.6
5	100	49/14	248.1	=	24810	11/0	12.2	=	1220	36.1	29.9
6	100	47/17	206.4	=	20640	10/1	12.1	=	1210	37.7	30.5
7	50	49/19	325.5	=	16275	13/1	16	=	800	37.8	30.1
8	25	49/22	387.3	=	9682.5	13/1	16	=	400	37.8	30.6
9	20	49/15	261.3	=	5226	9/2	12	=	240	37.3	30.7

Experiment 3

Table I-5: Experiment water characteristics

pH	Specific Conductance (µS/cm)	Total Hardness (mg/L $CaCO_3$)	Salinity (ppt)	TDS (mg/L)
8.3	1833	552	0.3	456

Table I-6: Quanti-Tray results and MPN for total coliform and E.coli

Bottle	Dilution	Total coliform	MPN	Total coliform MPN		*E.coli*	MPN	*E.coli* MPN		Temperature Light /Dark (°C)	
0	**500**	**49/19**	**325.5**	=	162750	**20/3**	**28.8**	=	14400	21	21
1	250	49/22	387.3	=	96825	26/3	39.9	=	9975	28.5	25.5
2	200	49/17	290.9	=	58180	27/2	40.4	=	8080	33.8	27.3
3	200	49/18	307.6	=	61520	16/1	20.1	=	4020	35.4	28.1
4	200	49/12	224.7	=	44940	10/1	12.1	=	2420	36.1	29.4
5	100	49/21	365.4	=	36540	18/4	26.9	=	2690	36.4	29.7
6	100	49/20	344.8	=	34480	10/0	11	=	1100	37.7	30.2
7	50	49/25	461.1	=	23055	19/2	25.9	=	1295	38	30.4
8	25	49/17	290.9	=	7272.5	11/0	12.2	=	305	38	30.7
9	20	49/24	435.2	=	8704	10/3	14.4	=	288	37.4	30.7

Appendix J: Datasheet from IDEXX for determining MPN

Quanti-Tray*/2000

Insert and Most Probable Number (MPN) Table

IDX 33/02 – 06/12

WATER ANALYSIS METHODS

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*Quanti-Tray and Colilert are trademarks or registered trademarks of IDEXX Laboratories, Inc. or its affiliates in the United States and/or other countries.

Patent information: idexx.com/patents.

One IDEXX Drive
Westbrook, Maine 04092 USA

Quanti-Tray*/2000

Introduction

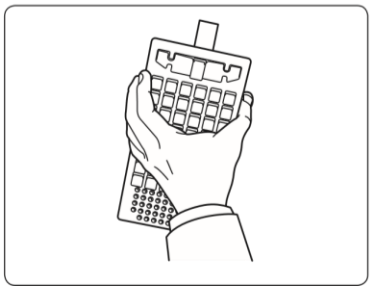
IDEXX Quanti-Tray*/2000 is designed to give quantitated bacterial counts of 100 mL samples using IDEXX reagent products. Add the reagent/sample mixture to a Quanti-Tray/2000, seal it in a Quanti-Tray* Sealer and incubate per the reagent instructions. Count the number of positive large and small wells and use the Most Probable Number (MPN) Table attached to determine the MPN.

Contents

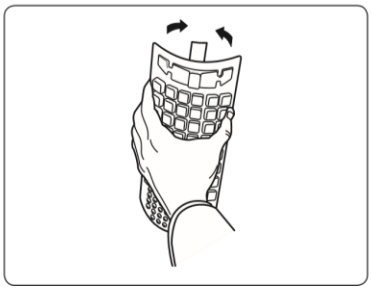
This package contains sterile Quanti-Tray/2000 trays.

User Instructions

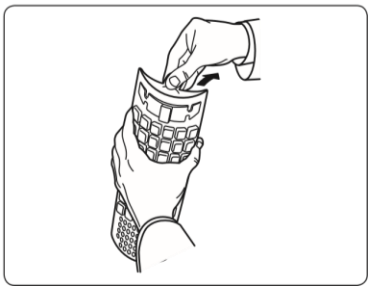
1. Use one hand to hold a Quanti-Tray upright with the well side facing the palm.



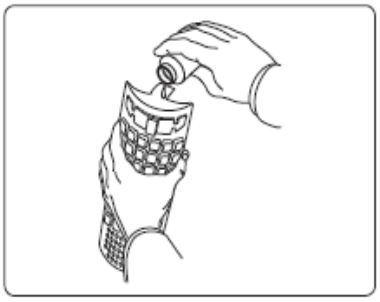
2. Squeeze the upper part of the Quanti-Tray so that the Quanti-Tray bends toward the palm.



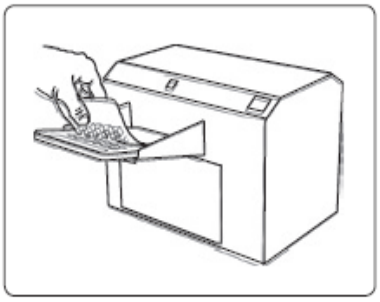
3. Gently pull foil tab to separate the foil from the tray. Avoid touching the inside of the foil or tray.



4. Pour the reagent/sample mixture directly into the Quanti-Tray, avoiding contact with the foil tab. Tap the small wells 2-3 times to release any air bubbles. Allow foam to settle.



5. Place the sample-filled Quanti-Tray onto the Quanti-Tray/2000 rubber insert of the Quanti-Tray Sealer with the well side (plastic) of the Quanti-Tray facing down.



6. Seal according to the Quanti-Tray Sealer instructions.
7. Incubate according to reagent instructions.
8. Count large and small positive wells and refer to the Quanti-Tray/2000 MPN table to find the MPN.†
9. Dispose of media in accordance with good laboratory practices.

For technical support, please call:
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Australia: + 1800 443 399
Idexx.com/water

One IDEXX Drive
Westbrook, Maine 04092 USA

IDEXX Quanti-Tray*/2000 MPN Table

# Large Wells Positive	# Small Wells Positive																								
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.8	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.6	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	158.5	163.1	167.9	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	167.0	172.5	178.2	184.2	190.4	196.8	203.5	210.5	217.8	225.4	233.3
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.4	214.2	222.4	231.0	240.0	249.5	259.5	270.0
48	123.9	128.4	133.1	137.9	143.0	148.3	153.9	159.7	165.8	172.2	178.9	186.0	193.5	201.4	209.8	218.7	228.2	238.2	248.9	260.3	272.3	285.1	298.7	313.0	328.2
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.9	248.1	261.3	275.5	290.9	307.6	325.5	344.8	365.4	387.3	410.6	435.2

IDEXX Quanti-Tray®/2000 MPN Table

# Large Wells Positive	# Small Wells Positive																							
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
0	25.3	26.4	27.4	28.4	29.5	30.5	31.5	32.6	33.6	34.7	35.7	36.8	37.8	38.9	40.0	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.5
1	26.6	27.7	28.7	29.8	30.8	31.9	32.9	34.0	35.0	36.1	37.2	38.2	39.3	40.4	41.4	42.5	43.6	44.7	45.7	46.8	47.9	49.0	50.1	51.2
2	27.9	29.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7	40.8	41.9	43.0	44.0	45.1	46.2	47.3	48.4	49.5	50.6	51.7	52.8
3	29.3	30.4	31.4	32.5	33.6	34.7	35.8	36.8	37.9	39.0	40.1	41.2	42.3	43.4	44.5	45.6	46.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5
4	30.7	31.8	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8	43.9	45.0	46.1	47.2	48.3	49.5	50.6	51.7	52.9	54.0	55.1	56.3
5	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.9	41.0	42.1	43.2	44.4	45.5	46.6	47.7	48.9	50.0	51.2	52.3	53.5	54.6	55.8	56.9	58.1
6	33.5	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.8	46.0	47.1	48.3	49.4	50.6	51.7	52.9	54.1	55.2	56.4	57.6	58.7	59.9
7	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.8	50.0	51.2	52.3	53.5	54.7	55.9	57.1	58.3	59.4	60.6	61.8
8	36.6	37.7	38.9	40.0	41.2	42.3	43.5	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.5	57.7	59.0	60.2	61.4	62.6	63.8
9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2	52.4	53.6	54.8	56.0	57.2	58.4	59.7	60.9	62.1	63.4	64.6	65.8
10	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.6	51.8	53.0	54.2	55.5	56.7	57.9	59.2	60.4	61.7	62.9	64.2	65.4	66.7	67.9
11	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.7	54.9	56.1	57.4	58.6	59.9	61.2	62.4	63.7	65.0	66.3	67.5	68.8	70.1
12	43.1	44.3	45.6	46.8	48.1	49.3	50.6	51.8	53.1	54.3	55.6	56.8	58.1	59.4	60.7	62.0	63.2	64.5	65.8	67.1	68.4	69.7	71.0	72.4
13	44.9	46.1	47.4	48.6	49.9	51.2	52.5	53.7	55.0	56.3	57.6	58.9	60.2	61.5	62.8	64.1	65.4	66.7	68.0	69.3	70.7	72.0	73.3	74.7
14	46.7	48.0	49.3	50.5	51.8	53.1	54.4	55.7	57.0	58.3	59.6	60.9	62.3	63.6	64.9	66.3	67.6	68.9	70.3	71.6	73.0	74.4	75.7	77.1
15	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1	64.5	65.8	67.2	68.5	69.9	71.3	72.6	74.0	75.4	76.8	78.2	79.6
16	50.5	51.8	53.2	54.5	55.8	57.2	58.5	59.9	61.2	62.6	64.0	65.3	66.7	68.1	69.5	70.9	72.3	73.7	75.1	76.5	77.9	79.3	80.8	82.2
17	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1	70.5	71.9	73.3	74.8	76.2	77.6	79.1	80.5	82.0	83.5	84.9
18	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	78.8	80.3	81.8	83.3	84.8	86.3	87.8
19	56.8	58.2	59.6	61.0	62.4	63.9	65.3	66.8	68.2	69.7	71.1	72.6	74.1	75.5	77.0	78.5	80.0	81.5	83.1	84.6	86.1	87.6	89.2	90.7
20	59.0	60.4	61.9	63.3	64.8	66.3	67.7	69.2	70.7	72.2	73.7	75.2	76.7	78.2	79.8	81.3	82.8	84.4	85.9	87.5	89.1	90.7	92.2	93.8
21	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	76.4	77.9	79.5	81.1	82.6	84.2	85.8	87.4	89.0	90.6	92.2	93.8	95.4	97.1
22	63.8	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8	82.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5
23	66.3	67.8	69.4	71.0	72.5	74.1	75.7	77.3	78.9	80.5	82.2	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	98.9	100.6	102.4	104.1
24	68.9	70.5	72.1	73.7	75.3	77.0	78.6	80.3	81.9	83.6	85.2	86.9	88.6	90.3	92.0	93.8	95.5	97.2	99.0	100.7	102.5	104.3	106.1	107.9
25	71.7	73.3	75.0	76.6	78.3	80.0	81.7	83.3	85.1	86.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	106.3	108.2	110.0	111.9
26	74.6	76.3	78.0	79.7	81.4	83.1	84.8	86.6	88.4	90.1	91.9	93.7	95.5	97.3	99.2	101.0	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2
27	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.5	97.4	99.3	101.2	103.1	105.0	106.9	108.8	110.8	112.7	114.7	116.7	118.7	120.7
28	80.8	82.6	84.4	86.3	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5	107.5	109.5	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8
30	87.8	89.7	91.7	93.6	95.6	97.6	99.6	101.6	103.7	105.7	107.8	109.9	112.0	114.2	116.3	118.5	120.6	122.8	125.1	127.3	129.5	131.8	134.1	136.4
31	91.6	93.6	95.6	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7	116.9	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5
32	95.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.8	129.2	131.6	134.0	136.5	139.0	141.5	144.0	146.6	149.1
33	100.0	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2	120.5	122.9	125.4	127.8	130.3	132.8	135.3	137.8	140.4	143.0	145.6	148.3	150.9	153.7	156.4
34	104.7	107.0	109.3	111.7	114.0	116.4	118.9	121.3	123.8	126.3	128.8	131.4	134.0	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	158.6	161.5	164.4
35	109.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.9	132.6	135.3	138.0	140.8	143.6	146.4	149.2	152.1	155.0	158.0	161.0	164.0	167.1	170.2	173.3
36	115.2	117.8	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.5	142.4	145.3	148.3	151.3	154.3	157.3	160.5	163.6	166.8	170.0	173.3	176.6	179.9	183.3
37	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.3	150.3	153.5	156.7	159.9	163.1	166.5	169.8	173.2	176.7	180.2	183.7	187.3	191.0	194.7
38	127.9	130.8	133.8	136.8	139.9	143.0	146.2	149.4	152.6	155.9	159.2	162.6	166.1	169.6	173.2	176.8	180.4	184.2	188.0	191.8	195.7	199.7	203.7	207.7
39	135.3	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1	176.9	180.7	184.7	188.7	192.7	196.8	201.0	205.3	209.6	214.0	218.5	223.0
40	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173.0	177.0	181.1	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	221.1	226.0	231.0	236.0	241.1
41	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.5	185.8	190.3	194.8	199.5	204.2	209.1	214.0	219.1	224.2	229.4	234.8	240.2	245.8	251.5	257.2	263.1
42	164.3	168.6	172.9	177.3	181.9	186.5	191.3	196.1	201.1	206.2	211.4	216.7	222.2	227.7	233.4	239.2	245.2	251.3	257.5	263.8	270.3	276.9	283.6	290.5
43	177.5	182.3	187.3	192.4	197.6	202.9	208.4	214.0	219.8	225.8	231.8	238.1	244.5	251.0	257.7	264.6	271.7	278.9	286.3	293.8	301.5	309.4	317.4	325.7
44	193.6	199.3	205.1	211.0	217.2	223.5	230.0	236.7	243.6	250.8	258.1	265.6	273.3	281.2	289.4	297.8	306.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4
45	214.1	220.9	227.9	235.2	242.7	250.4	258.4	266.7	275.3	284.1	293.3	302.6	312.3	322.3	332.5	343.0	353.8	364.9	376.2	387.9	399.8	412.0	424.5	437.4
46	241.5	250.0	258.9	268.2	277.8	287.8	298.1	308.8	319.9	331.4	343.3	355.5	368.1	381.1	394.5	408.3	422.5	437.1	452.0	467.4	483.3	499.6	516.3	533.5
47	280.9	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.8	436.6	454.1	472.1	490.7	509.9	529.8	550.4	571.7	593.8	616.7	640.5	665.3	691.0
48	344.1	360.9	378.4	396.8	416.0	436.0	456.9	478.6	501.2	524.7	549.3	574.8	601.5	629.4	658.6	689.3	721.5	755.6	791.5	829.7	870.4	913.9	960.6	1011.2
49	461.1	488.4	517.2	547.5	579.4	613.1	648.8	686.7	727.0	770.1	816.4	866.4	920.8	980.4	1046.2	1119.9	1203.3	1299.7	1413.6	1553.1	1732.9	1986.3	2419.6	>2419.6